NOVEL METHODS OF CONSTRUCTING LIBRARIES COMPRISING DISPLAYED AND/OR EXPRESSED MEMBERS OF A DIVERSE FAMILY OF PEPTIDES, POLYPEPTIDES OR PROTEINS AND THE NOVEL LIBRARIES

This application is a continuation-in-part of United States provisional application 06/198,069, filed April 17, 2000, a continuation-in-part of United States patent application 09/837,306, filed on April 17, 2001, and a continuation-in-part of United States application XX/XXX,XXX, filed by Express Mail(EI125454535US) on October 25, 2001. All of the earlier applications are specifically incorporated by reference herein.

The present invention relates to libraries of genetic packages that display and/or express a member of a diverse family of peptides, polypeptides or proteins and collectively display and/or express at least a portion of the diversity of the family. In an alternative embodiment, the invention relates to libraries that include a member of a diverse family of peptides, polypeptides or proteins and collectively comprise at least a portion of the diversity of the family. In a preferred embodiment, the displayed and/or expressed polypeptides are human Fabs.

More specifically, the invention is directed to the methods of cleaving single-stranded nucleic acids at chosen locations, the cleaved nucleic acids encoding, at least in part, the peptides, polypeptides

or proteins displayed on the genetic packages of, and/or expressed in, the libraries of the invention. In a preferred embodiment, the genetic packages are filamentous phage or phagemids or yeast.

The present invention further relates to vectors for displaying and/or expressing a diverse family of peptides, polypeptides or proteins.

The present invention further relates to methods of screening the libraries of the invention and to the peptides, polypeptides and proteins identified by such screening.

BACKGROUND OF THE INVENTION

It is now common practice in the art to prepare libraries of genetic packages that display, express or comprise a member of a diverse family of peptides, polypeptides or proteins and collectively display, express or comprise at least a portion of the diversity of the family. In many common libraries, the peptides, polypeptides or proteins are related to antibodies. Often, they are Fabs or single chain antibodies.

In general, the DNAs that encode members of the families to be displayed and/or expressed must be amplified before they are cloned and used to display and/or express the desired member. Such amplification typically makes use of forward and backward primers.

Such primers can be complementary to sequences native to the DNA to be amplified or complementary to oligonucleotides attached at the 5' or 3' ends of that DNA. Primers that are complementary to sequences native to the DNA to be amplified are disadvantaged in that they bias the members of the

families to be displayed. Only those members that contain a sequence in the native DNA that is substantially complementary to the primer will be amplified. Those that do not will be absent from the family. For those members that are amplified, any diversity within the primer region will be suppressed.

For example, in European patent 368,684 B1, the primer that is used is at the 5' end of the V_H region of an antibody gene. It anneals to a sequence region in the native DNA that is said to be "sufficiently well conserved" within a single species. Such primer will bias the members amplified to those having this "conserved" region. Any diversity within this region is extinguished.

It is generally accepted that human antibody genes arise through a process that involves a combinatorial selection of V and J or V, D, and J followed by somatic mutations. Although most diversity occurs in the Complementary Determining Regions (CDRs), diversity also occurs in the more conserved Framework Regions (FRs) and at least some of this diversity confers or enhances specific binding to antigens (Ag). As a consequence, libraries should contain as much of the CDR and FR diversity as possible.

To clone the amplified DNAs of the peptides, polypeptides or proteins that they encode for display on a genetic package and/or for expression, the DNAs must be cleaved to produce appropriate ends for ligation to a vector. Such cleavage is generally effected using restriction endonuclease recognition sites carried on the primers. When the primers are at the 5' end of DNA produced from reverse transcription of RNA, such restriction leaves deleterious 5' untranslated regions in the amplified DNA. These

regions interfere with expression of the cloned genes and thus the display of the peptides, polypeptides and proteins coded for by them.

SUMMARY OF THE INVENTION

It is an object of this invention to provide novel methods for constructing libraries that display, express or comprise a member of a diverse family of peptides, polypeptides or proteins and collectively display, express or comprise at least a portion of the diversity of the family. These methods are not biased toward DNAs that contain native sequences that are complementary to the primers used for amplification. They also enable any sequences that may be deleterious to expression to be removed from the amplified DNA before cloning and displaying and/or expressing.

It is another object of this invention to provide a method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

(i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

It is a further object of this invention to provide an alternative method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(ii) cleaving the nucleic acid solely at the cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur

at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

In an alternative embodiment of this object of the invention, the restriction endonuclease recognition site is not initially located in the double-stranded part of the oligonucleotide. Instead, it is part of an amplification primer, which primer is complementary to the double-stranded region of the oligonucleotide. On amplification of the DNA-partially double-stranded combination, the restriction endonuclease recognition site carried on the primer becomes part of the DNA. It can then be used to cleave the DNA.

Preferably, the restriction endonuclease recognition site is that of a Type II-S restriction endonuclease whose cleavage site is located at a known distance from its recognition site.

It is another object of the present invention to provide a method of capturing DNA molecules that comprise a member of a diverse family of DNAs and collectively comprise at least a portion of the diversity of the family. These DNA molecules in single-stranded form have been cleaved by one of the methods of this invention. This method involves ligating the individual single-stranded DNA members of the family to a partially duplex DNA complex. The method comprises the steps of:

(i) contacting a single-stranded nucleic acid sequence that has been cleaved with a restriction endonuclease with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic

acid in the region that remains after cleavage, the double-stranded region of the oligonucleotide including any sequences necessary to return the sequences that remain after cleavage into proper reading frame for expression and containing a restriction endonuclease recognition site 5' of those sequences; and

(ii) cleaving the partially double-stranded oligonucleotide sequence solely at the restriction endonuclease cleavage site contained within the double-stranded region of the partially double-stranded oligonucleotide.

As before, in this object of the invention, the restriction endonuclease recognition site need not be located in the double-stranded portion of the oligonucleotide. Instead, it can be introduced on amplification with an amplification primer that is used to amplify the DNA-partially double-stranded oligonucleotide combination.

It is another object of this invention to prepare libraries, that display, express or comprise a diverse family of peptides, polypeptides or proteins and collectively display, express or comprise at least part of the diversity of the family, using the methods and DNAs described above.

It is an object of this invention to screen those libraries to identify useful peptides, polypeptides and proteins and to use those substances in human therapy.

Additional objects of the invention are reflected in claims 1-116. Each of these claims is

specifically incorporated by reference in this specification.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of various methods that may be employed to amplify VH genes without using primers specific for VH sequences.

FIG. 2 is a schematic of various methods that may be employed to amplify VL genes without using primers specific for VL sequences.

FIG. 3 is a schematic of RACE amplification of antibody heavy and light chains.

FIG. 4 depicts gel analysis of amplification products obtained after the primary PCR reaction from 4 different patient samples.

FIG. 5 depicts gel analysis of cleaved kappa DNA from Example 2.

FIG. 6 depicts gel analysis of extender-cleaved kappa DNA from Example 2.

FIG. 7 depicts gel analysis of the PCR product from the extender-kappa amplification from Example 2.

FIG. 8 depicts gel analysis of purified PCR product from the extender-kappa amplification from Example 2.

FIG. 9 depicts gel analysis of cleaved and ligated kappa light chains from Example 2.

FIG. 10 is a schematic of the design for CDR1 and CDR2 synthetic diversity.

FIG. 11 is a schemaitc of the cloning schedule for construction of the heavy chain repertoire.

FIG. 12 is a schematic of the cleavage and ligation of the antibody light chain.

FIG. 13 depicts gel analysis of cleaved and ligated lambda light chains from Example 4.

FIG. 14 is a schematic of the cleavage and ligation of the antibody heavy chain.

FIG. 15 depicts gel analysis of cleaved and ligated lambda light chains from Example 5.

FIG. 16 is a schematic of a phage display vector.

FIG. 17 is a schematic of a Fab cassette.

FIG. 18 is a schematic of a process for incorporating fixed FR1 residues in an antibody lambda sequence.

FIG. 19 is a schematic of a process for incorporating fixed FR1 residues in an antibody kappa sequence.

FIG. 20 is a schematic of a process for incorporating fixed FR1 residues in an antibody heavy chain sequence.

TERMS

In this application, the following terms and abbreviations are used:

Sense strand	The upper strand of ds DNA as usually written. In the sense strand, 5'-ATG-3' codes for Met.
Antisense strand	The lower strand of ds DNA as usually written. In the antisense strand, 3'-TAC-5' would correspond to a Met codon in the sense strand.

Forward primer	A "forward" primer is complementary to a part of the sense strand and primes for synthesis of a new antisense-strand molecule. "Forward primer" and "lower-strand primer" are equivalent.
Backward primer	A "backward" primer is complementary to a part of the antisense strand and primes for synthesis of a new sense-strand molecule. "Backward primer" and "top-strand primer" are equivalent.
Bases	Bases are specified either by their position in a vector or gene as their position within a gene by codon and base. For example, "89.1" is the first base of codon 89, 89.2 is the second base of codon 89.
Sv	Streptavidin
Ap	Ampicillin
ap^R	A gene conferring ampicillin resistance.
RERS	Restriction endonuclease recognition site

RE	Restriction endonuclease - cleaves preferentially at RERS
URE	Universal restriction endonuclease
Functionally complementary	Two sequences are sufficiently complementary so as to anneal under the chosen conditions.
AA	Amino acid
PCR	Polymerization chain reaction
GLGs	Germline genes
Ab	Antibody: an immunoglobin. The term also covers any protein having a binding domain which is homologous to an immunoglobin binding domain. A few examples of antibodies within this definition are, *inter alia*, immunoglobin isotypes and the Fab, $F(ab^1)_2$, scfv, Fv, dAb and Fd fragments.
Fab	Two chain molecule comprising an Ab light chain and part of a heavy-chain.

scFv	A single-chain Ab comprising either VH::linker::VL or VL::linker::VH
w.t.	Wild type
HC	Heavy chain
LC	Light chain
VK	A variable domain of a Kappa light chain.
VH	A variable domain of a heavy chain.
VL	A variable domain of a lambda light chain.

In this application when it is said that nucleic acids are cleaved solely at the cleavage site of a restriction endonuclease, it should be understood that minor cleavage may occur at random, *e.g.*, at non-specific sites other than the specific cleavage site that is characteristic of the restriction endonuclease. The skilled worker will recognize that such non-specific, random cleavage is the usual occurrence. Accordingly, "solely at the cleavage site" of a restriction endonuclease means that cleavage occurs preferentially at the site characteristic of that endonuclease.

As used in this application and claims, the term "cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the

oligonucleotide" includes cleavage sites formed by the single-stranded portion of the partially double-stranded ologonucleotide duplexing with the single-stranded DNA, cleavage sites in the double-stranded portion of the partially double-stranded oligonucleotide, and cleavage sites introduced by the amplification primer used to amplify the single-stranded DNA-partially double-stranded oligonucleotide combination.

In the two methods of this invention for preparing single-stranded nucleic acid sequences, the first of those cleavage sites is preferred. In the methods of this invention for capturing diversity and cloning a family of diverse nucleic acid sequences, the latter two cleavage sites are preferred.

In this application, all references referred to are specifically incorporated by reference.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The nucleic acid sequences that are useful in the methods of this invention, *i.e.*, those that encode at least in part the individual peptides, polypeptides and proteins displayed, or expressed in or comprising the libraries of this invention, may be native, synthetic or a combination thereof. They may be mRNA, DNA or cDNA. In the preferred embodiment, the nucleic acids encode antibodies. Most preferably, they encode Fabs.

The nucleic acids useful in this invention may be naturally diverse, synthetic diversity may be introduced into those naturally diverse members, or the diversity may be entirely synthetic. For example, synthetic diversity can be introduced into one or more CDRs of antibody genes. Preferably, it is introduced

into CDR1 and CDR2 of immunoglobulins. Preferably, natural diversity is captured in the CDR3 regions of the immunoglogin genes of this invention from B cells. Most preferably, the nucleic acids of this invention comprise a population of immunoglobin genes that comprise synthetic diversity in at least one, and more preferably both of the CDR1 and CDR2 and diversity in CDR3 captured from B cells.

Synthetic diversity may be created, for example, through the use of TRIM technology (U.S. 5,869,644). TRIM technology allows control over exactly which amino-acid types are allowed at variegated positions and in what proportions. In TRIM technology, codons to be diversified are synthesized using mixtures of trinucleotides. This allows any set of amino acid types to be included in any proportion.

Another alternative that may be used to generate diversified DNA is mixed oligonucleotide synthesis. With TRIM technology, one could allow Ala and Trp. With mixed oligonucleotide synthesis, a mixture that included Ala and Trp would also necessarily include Ser and Gly. The amino-acid types allowed at the variegated positions are picked with reference to the structure of antibodies, or other peptides, polypeptides or proteins of the family, the observed diversity in germline genes, the observed somatic mutations frequently observed, and the desired areas and types of variegation.

In a preferred embodiment of this invention, the nucleic acid sequences for at least one CDR or other region of the peptides, polypeptides or proteins of the family are cDNAs produced by reverse transcription from mRNA. More preferably, the mRNAs are obtained from peripheral blood cells, bone marrow

cells, spleen cells or lymph node cells (such as B-lymphocytes or plasma cells) that express members of naturally diverse sets of related genes. More preferable, the mRNAs encode a diverse family of antibodies. Most preferably, the mRNAs are obtained from patients suffering from at least one autoimmune disorder or cancer. Preferably, mRNAs containing a high diversity of autoimmune diseases, such as systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome and vasculitis are used.

In a preferred embodiment of this invention, the cDNAs are produced from the mRNAs using reverse transcription. In this preferred embodiment, the mRNAs are separated from the cell and degraded using standard methods, such that only the full length (*i.e.*, capped) mRNAs remain. The cap is then removed and reverse transcription used to produce the cDNAs.

The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, *e.g.*, HJ de Haard et al., Journal of Biological Chemistry, 274(26):18218-30 (1999). In the preferred embodiment of this invention where the mRNAs encode antibodies, primers that are complementary to the constant regions of antibody genes may be used. Those primers are useful because they do not generate bias toward subclasses of antibodies. In another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes). Alternatively, sequences complementary to the primer may be attached to the termini of the antisense strand.

In one preferred embodiment of this invention, the reverse transcriptase primer may be biotinylated, thus allowing the cDNA product to be

immobilized on streptavidin (Sv) beads. Immobilization can also be effected using a primer labeled at the 5' end with one of a) free amine group, b) thiol, c) carboxylic acid, or d) another group not found in DNA that can react to form a strong bond to a known partner on an insoluble medium. If, for example, a free amine (preferably primary amine) is provided at the 5' end of a DNA primer, this amine can be reacted with carboxylic acid groups on a polymer bead using standard amide-forming chemistry. If such preferred immobilization is used during reverse transcription, the top strand RNA is degraded using well-known enzymes, such as a combination of RNAseH and RNAseA, either before or after immobilization.

The nucleic acid sequences useful in the methods of this invention are generally amplified before being used to display and/or express the peptides, polypeptides or proteins that they encode. Prior to amplification, the single-stranded DNAs may be cleaved using either of the methods described before. Alternatively, the single-stranded DNAs may be amplified and then cleaved using one of those methods.

Any of the well known methods for amplifying nucleic acid sequences may be used for such amplification. Methods that maximize, and do not bias, diversity are preferred. In a preferred embodiment of this invention where the nucleic acid sequences are derived from antibody genes, the present invention preferably utilizes primers in the constant regions of the heavy and light chain genes and primers to a synthetic sequence that are attached at the 5' end of the sense strand. Priming at such synthetic sequence avoids the use of sequences within the variable regions of the antibody genes. Those variable region priming

sites generate bias against V genes that are either of rare subclasses or that have been mutated at the priming sites. This bias is partly due to suppression of diversity within the primer region and partly due to lack of priming when many mutations are present in the region complementary to the primer. The methods disclosed in this invention have the advantage of not biasing the population of amplified antibody genes for particular V gene types.

The synthetic sequences may be attached to the 5' end of the DNA strand by various methods well known for ligating DNA sequences together. RT CapExtention is one preferred method.

In RT CapExtention (derived from Smart PCR(TM)), a short overlap (5'-...GGG-3' in the upper-strand primer (USP-GGG) complements 3'-CCC....5' in the lower strand) and reverse transcriptases are used so that the reverse complement of the upper-strand primer is attached to the lower strand.

FIGs. 1 and 2 show schematics to amplify VH and VL genes using RT CapExtention. FIG. 1 shows a schematic of the amplification of VH genes. FIG. 1, Panel A shows a primer specific to the poly-dT region of the 3' UTR priming synthesis of the first, lower strand. Primers that bind in the constant region are also suitable. Panel B shows the lower strand extended at its 3' end by three Cs that are not complementary to the mRNA. Panel C shows the result of annealing a synthetic top-strand primer ending in three GGGs that hybridize to the 3' terminal CCCs and extending the reverse transcription extending the lower strand by the reverse complement of the synthetic primer sequence. Panel D shows the result of PCR amplification using a 5' biotinylated synthetic top-strand primer that

replicates the 5' end of the synthetic primer of panel C and a bottom-strand primer complementary to part of the constant domain. Panel E shows immobilized double-stranded (ds) cDNA obtained by using a 5'-biotinylated top-strand primer.

FIG. 2 shows a similar schematic for amplification of VL genes. FIG. 2, Panel A shows a primer specific to the constant region at or near the 3' end priming synthesis of the first, lower strand. Primers that bind in the poly-dT region are also suitable. Panel B shows the lower strand extended at its 3' end by three Cs that are not complementary to the mRNA. Panel C shows the result of annealing a synthetic top-strand primer ending in three GGGs that hybridize to the 3' terminal CCCs and extending the reverse transcription extending the lower strand by the reverse complement of the synthetic primer sequence. Panel D shows the result of PCR amplification using a 5' biotinylated synthetic top-strand primer that replicates the 5' end of the synthetic primer of panel C and a bottom-strand primer complementary to part of the constant domain. The bottom-strand primer also contains a useful restriction endonuclease site, such as *Asc*I. Panel E shows immobilized ds cDNA obtained by using a 5'-biotinylated top-strand primer.

In FIGs. 1 and 2, each V gene consists of a 5' untranslated region (UTR) and a secretion signal, followed by the variable region, followed by a constant region, followed by a 3' untranslated region (which typically ends in poly-A). An initial primer for reverse transcription may be complementary to the constant region or to the poly A segment of the 3'-UTR. For human heavy-chain genes, a primer of 15 T is preferred. Reverse transcriptases attach several C

residues to the 3' end of the newly synthesized DNA. RT CapExtention exploits this feature. The reverse transcription reaction is first run with only a lower-strand primer. After about 1 hour, a primer ending in GGG (USP-GGG) and more RTase are added. This causes the lower-strand cDNA to be extended by the reverse complement of the USP-GGG up to the final GGG. Using one primer identical to part of the attached synthetic sequence and a second primer complementary to a region of known sequence at the 3' end of the sense strand, all the V genes are amplified irrespective of their V gene subclass.

In another preferred embodiment, synthetic sequences may be added by Rapid Amplification of cDNA Ends (RACE) (see Frohman, M.A., Dush, M.K., & Martin, G.R. (1988) Proc. Natl. Acad. Sci. USA (85): 8998-9002).

FIG. 1 shows a schematic of RACE amplification of antibody heavy and light chains. First, mRNA is selected by treating total or poly(A+) RNA with calf intestinal phosphatase (CIP) to remove the 5'-phosphate from all molecules that have them such as ribosomal RNA, fragmented mRNA, tRNA and genomic DNA. Full length mRNA (containing a protective 7-methyl cap structure) is uneffected. The RNA is then treated with tobacco acid pyrophosphatase (TAP) to remove the cap structure from full length mRNAs leaving a 5'-monophosphate group. Next, a synthetic RNA adaptor is ligated to the RNA population, only molecules which have a 5-phosphate (uncapped, full length mRNAs) will accept the adaptor. Reverse trascriptase reactions using an oligodT primer, and nested PCR (using one adaptor primer (located in the 5'

synthetic adaptor) and one primer for the gene) are then used to amplify the desired transcript.

In a preferred embodiment of this invention, the upper strand or lower strand primer may be also biotinylated or labeled at the 5' end with one of a) free amino group, b) thiol, c) carboxylic acid and d) another group not found in DNA that can react to form a strong bond to a known partner as an insoluble medium. These can then be used to immobilize the labeled strand after amplification. The immobilized DNA can be either single or double-stranded.

After amplification (using *e.g.*, RT CapExtension or RACE), the DNAs of this invention are rendered single-stranded. For example, the strands can be separated by using a biotinylated primer, capturing the biotinylated product on streptavidin beads, denaturing the DNA, and washing away the complementary strand. Depending on which end of the captured DNA is wanted, one will choose to immobilize either the upper (sense) strand or the lower (antisense) strand.

To prepare the single-stranded amplified DNAs for cloning into genetic packages so as to effect display of, or for expression of, the peptides, polypeptides or proteins encoded, at least in part, by those DNAs, they must be manipulated to provide ends suitable for cloning and display and/or expression. In particular, any 5' untranslated regions and mammalian signal sequences must be removed and replaced, in frame, by a suitable signal sequence that functions in the display or expression host. Additionally, parts of the variable domains (in antibody genes) may be removed and replaced by synthetic segments containing synthetic diversity. The diversity of other gene families may likewise be expanded with synthetic diversity.

According to the methods of this invention, there are two ways to manipulate the single-stranded DNAs for display and/or expression. The first method comprises the steps of:

(i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

In this first method, short oligonucleotides are annealed to the single-stranded DNA so that restriction endonuclease recognition sites formed within the now locally double-stranded regions of the DNA can be cleaved. In particular, a recognition site

that occurs at the same position in a substantial fraction of the single-stranded DNAs is identical.

For antibody genes, this can be done using a catalog of germline sequences. See, *e.g.*, "http://www.mrc-cpe.cam.ac.uk/imt-doc/restricted/ok.html." Updates can be obtained from this site under the heading "Amino acid and nucleotide sequence alignments." For other families, similar comparisons exist and may be used to select appropriate regions for cleavage and to maintain diversity.

For example, Table 1 depicts the DNA sequences of the FR3 regions of the 51 known human VH germline genes. In this region, the genes contain restriction endonuclease recognition sites shown in Table 2. Restriction endonucleases that cleave a large fraction of germline genes at the same site are preferred over endonucleases that cut at a variety of sites. Furthermore, it is preferred that there be only one site for the restriction endonucleases within the region to which the short oligonucleotide binds on the single-stranded DNA, *e.g.*, about 10 bases on either side of the restriction endonuclease recognition site.

An enzyme that cleaves downstream in FR3 is also more preferable because it captures fewer mutations in the framework. This may be advantageous is some cases. However, it is well known that framework mutations exist and confer and enhance antibody binding. The present invention, by choice of appropriate restriction site, allows all or part of FR3 diversity to be captured. Hence, the method also allows extensive diversity to be captured.

Finally, in the methods of this invention restriction endonucleases that are active between about 37°C and about 75°C are used. Preferably, restriction

endonucleases that are active between about 45°C and about 75°C may be used. More preferably, enzymes that are active above 50°C, and most preferably active about 55°C, are used. Such temperatures maintain the nucleic acid sequence to be cleaved in substantially single-stranded form.

Enzymes shown in Table 2 that cut many of the heavy chain FR3 germline genes at a single position include: *Mae*III(24@4), *Tsp*45I(21@4), *Hph*I(44@5), *Bsa*JI(23@65), *Alu*I(23@47), *Blp*I(21@48), *Dde*I(29@58), *Bgl*II(10@61), *Msl*I(44@72), *Bsi*EI(23@74), *Eae*I(23@74), *Eag*I(23@74), *Hae*III(25@75), *Bst*4CI(51@86), *Hpy*CH4III(51@86), *Hinf*I(38@2), *Mly*I(18@2), *Ple*I(18@2), *Mnl*I(31@67), *Hpy*CH4V(21@44), *Bsm*AI(16@11), *Bpm*I(19@12), *Xmn*I(12@30), and *Sac*I(11@51). (The notation used means, for example, that *Bsm*AI cuts 16 of the FR3 germline genes with a restriction endonuclease recognition site beginning at base 11 of FR3.)

For cleavage of human heavy chains in FR3, the preferred restriction endonucleases are: *Bst*4CI (or *Taa*I or *Hpy*CH4III), *Blp*I, *Hpy*CH4V, and *Msl*I. Because ACNGT (the restriction endonuclease recognition site for *Bst*4CI, *Taa*I, and *Hpy*CH4III) is found at a consistent site in all the human FR3 germline genes, one of those enzymes is the most preferred for capture of heavy chain CDR3 diversity. *Blp*I and *Hpy*CH4V are complementary. *Blp*I cuts most members of the VH1 and VH4 families while *Hpy*CH4V cuts most members of the VH3, VH5, VH6, and VH7 families. Neither enzyme cuts VH2s, but this is a very small family, containing only three members. Thus, these enzymes may also be used in preferred embodiments of the methods of this invention.

The restriction endonucleases *Hpy*CH4III, *Bst*4CI, and *Taa*I all recognize 5'-ACnGT-3' and cut upper strand DNA after n and lower strand DNA before the base complementary to n. This is the most preferred restriction endonuclease recognition site for this method on human heavy chains because it is found in all germline genes. Furthermore, the restriction endonuclease recognition region (ACnGT) matches the second and third bases of a tyrosine codon (t<u>a</u>y) and the following cysteine codon (t<u>g</u>y) as shown in Table 3. These codons are highly conserved, especially the cysteine in mature antibody genes.

Table 4 E shows the distinct oligonucleotides of length 22 (except the last one which is of length 20) bases. Table 5 C shows the analysis of 1617 actual heavy chain antibody genes. Of these, 1511 have the site and match one of the candidate oligonucleotides to within 4 mismatches. Eight oligonucleotides account for most of the matches and are given in Table 4 F.1. The 8 oligonucleotides are very similar so that it is likely that satisfactory cleavage will be achieved with only one oligonucleotide (such as H43.77.97.1-02#1) by adjusting temperature, pH, salinity, and the like. One or two oligonucleotides may likewise suffice whenever the germline gene sequences differ very little and especially if they differ very little close to the restriction endonuclease recognition region to be cleaved. Table 5 D shows a repeat analysis of 1617 actual heavy chain antibody genes using only the 8 chosen oligonucleotides. This shows that 1463 of the sequences match at least one of the oligonucleotides to within 4 mismatches and have the site as expected.

Only 7 sequences have a second *Hpy*CH4III restriction endonuclease recognition region in this region.

Another illustration of choosing an appropriate restriction endonuclease recognition site involves cleavage in FR1 of human heavy chains. Cleavage in FR1 allows capture of the entire CDR diversity of the heavy chain.

The germline genes for human heavy chain FR1 are shown in Table 6. Table 7 shows the restriction endonuclease recognition sites found in human germline genes FR1s. The preferred sites are *Bsg*I(GTGCAG;39@4), *Bso*FI(GCngc;43@6,11@9,2@3,1@12), *Tse*I(Gcwgc;43@6,11@9,2@3,1@12), *Msp*A1I(CMGckg;46@7,2@1), *Pvu*II(CAGctg;46@7,2@1), *Alu*I(AGct;48@82@2), *Dde*I(Ctnag;22@52,9@48), *Hph*I(tcacc;22@80), *Bss*KI(Nccngg;35@39,2@40), *Bsa*JI(Ccnngg;32@40,2@41), *Bst*NI(CCwgg;33@40), *Scr*FI(CCngg;35@40,2@41), *Eco*O109I(RGgnccy;22@46, 11@43), *Sau*96I(Ggncc;23@47,11@44), AvaII(Ggwcc;23@47,4@44), *Ppu*MI(RGgwccy;22@46,4@43), *Bsm*FI(gtccc;20@48), *Hin*fI(Gantc;34@16,21@56,21@77), *Tfi*I(21@77), *Mly*I(GAGTC;34@16), *Mly*I(gactc;21@56), and *Alw*NI(CAGnnnctg;22@68). The more preferred sites are *MspAI* and *PvuII*. *Msp*AI and *Pvu*II have 46 sites at 7-12 and 2 at 1-6. To avoid cleavage at both sites, oligonucleotides are used that do not fully cover the site at 1-6. Thus, the DNA will not be cleaved at that site. We have shown that DNA that extends 3, 4, or 5 bases beyond a *Pvu*II-site can be cleaved efficiently.

Another illustration of choosing an appropriate restriction endonuclease recognition site involves cleavage in FR1 of human kappa light chains. Table 8 shows the human kappa FR1 germline genes and

Table 9 shows restriction endonuclease recognition sites that are found in a substantial number of human kappa FR1 germline genes at consistent locations. Of the restriction endonuclease recognition sites listed, *Bsm*AI and *Pfl*FI are the most preferred enzymes. *Bsm*AI sites are found at base 18 in 35 of 40 germline genes. *Pfl*FI sites are found in 35 of 40 germline genes at base 12.

Another example of choosing an appropriate restriction endonuclease recognition site involves cleavage in FR1 of the human lambda light chain. Table 10 shows the 31 known human lambda FR1 germline gene sequences. Table 11 shows restriction endonuclease recognition sites found in human lambda FR1 germline genes. *Hin*fI and *Dde*I are the most preferred restriction endonucleases for cutting human lambda chains in FR1.

After the appropriate site or sites for cleavage are chosen, one or more short oligonucleotides are prepared so as to functionally complement, alone or in combination, the chosen recognition site. The oligonucleotides also include sequences that flank the recognition site in the majority of the amplified genes. This flanking region allows the sequence to anneal to the single-stranded DNA sufficiently to allow cleavage by the restriction endonuclease specific for the site chosen.

The actual length and sequence of the oligonucleotide depends on the recognition site and the conditions to be used for contacting and cleavage. The length must be sufficient so that the oligonucleotide is functionally complementary to the single-stranded DNA over a large enough region to allow the two strands

to associate such that cleavage may occur at the chosen temperature and at the desired location.

Typically, the oligonucleotides of this preferred method of the invention are about 17 to about 30 nucleotides in length. Below about 17 bases, annealing is too weak and above 30 bases there can be a loss of specificity. A preferred length is 18 to 24 bases.

Oligonucleotides of this length need not be identical complements of the germline genes. Rather, a few mismatches taken may be tolerated. Preferably, however, no more than 1-3 mismatches are allowed. Such mismatches do not adversely affect annealing of the oligonucleotide to the single-stranded DNA. Hence, the two DNAs are said to be functionally complementary.

The second method to manipulate the single-stranded DNAs of this invention for display and/or expression comprises the steps of:

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(ii) cleaving the nucleic acid solely at the cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

As explained above, the cleavage site may be formed by the single-stranded portion of the partially double-stranded oligonucleotide duplexing with the single-stranded DNA, the cleavage site may be carried in the double-stranded portion of the partially double-stranded oligonucleotide, or the cleavage site may be introduced by the amplification primer used to amplify the single-stranded DNA-partially double-stranded oligonucleotide combination. In this embodiment, the first is preferred. And, the restriction endonuclease recognition site may be located in either the double-stranded portion of the oligonucleotide or introduced by the amplification primer, which is complementary to that double-stranded region, as used to amplify the combination.

Preferably, the restriction endonuclease site is that of a Type II-S restriction endonuclease, whose cleavage site is located at a known distance from its recognition site.

This second method, preferably, employs Universal Restriction Endonucleases ("URE"). UREs are partially double-stranded oligonucleotides. The single-stranded portion or overlap of the URE consists of a DNA adapter that is functionally complementary to the sequence to be cleaved in the single-stranded DNA.

The double-stranded portion consists of a restriction endonuclease recognition site, preferably type II-S.

The URE method of this invention is specific and precise and can tolerate some (*e.g.*, 1-3) mismatches in the complementary regions, *i.e.*, it is functionally complementary to that region. Further, conditions under which the URE is used can be adjusted so that most of the genes that are amplified can be cut, reducing bias in the library produced from those genes.

The sequence of the single-stranded DNA adapter or overlap portion of the URE typically consists of about 14-22 bases. However, longer or shorter adapters may be used. The size depends on the ability of the adapter to associate with its functional complement in the single-stranded DNA and the temperature used for contacting the URE and the single-stranded DNA at the temperature used for cleaving the DNA with the restriction enzyme. The adapter must be functionally complementary to the single-stranded DNA over a large enough region to allow the two strands to associate such that the cleavage may occur at the chosen temperature and at the desired location. We prefer singe-stranded or overlap portions of 14-17 bases in length, and more preferably 18-20 bases in length.

The site chosen for cleavage using the URE is preferably one that is substantially conserved in the family of amplified DNAs. As compared to the first cleavage method of this invention, these sites do not need to be endonuclease recognition sites. However, like the first method, the sites chosen can be synthetic rather than existing in the native DNA. Such sites may be chosen by references to the sequences of

known antibodies or other families of genes. For example, the sequences of many germline genes are reported at http://www.mrc-cpe.cam.ac.uk/imt-doc/restricted/ok.html. For example, one preferred site occurs near the end of FR3 -- codon 89 through the second base of codon 93. CDR3 begins at codon 95.

The sequences of 79 human heavy-chain genes are also available at http://www.ncbi.nlm.nih.gov/entre2/nucleotide.html. This site can be used to identify appropriate sequences for URE cleavage according to the methods of this invention. *See, e.g.*, Table 12B.

Most preferably, one or more sequences are identified using these sites or other available sequence information. These sequences together are present in a substantial fraction of the amplified DNAs. For example, multiple sequences could be used to allow for known diversity in germline genes or for frequent somatic mutations. Synthetic degenerate sequences could also be used. Preferably, a sequence(s) that occurs in at least 65% of genes examined with no more than 2-3 mismatches is chosen

URE single-stranded adapters or overlaps are then made to be complementary to the chosen regions. Conditions for using the UREs are determined empirically. These conditions should allow cleavage of DNA that contains the functionally complementary sequences with no more than 2 or 3 mismatches but that do not allow cleavage of DNA lacking such sequences.

As described above, the double-stranded portion of the URE includes an endonuclease recognition site, preferably a Type II-S recognition site. Any enzyme that is active at a temperature necessary to maintain the single-stranded DNA substantially in that

form and to allow the single-stranded DNA adapter portion of the URE to anneal long enough to the single-stranded DNA to permit cleavage at the desired site may be used.

The preferred Type II-S enzymes for use in the URE methods of this invention provide asymmetrical cleavage of the single-stranded DNA. Among these are the enzymes listed in Table 13. The most preferred Type II-S enzyme is *FokI*.

When the preferred *FokI* containing URE is used, several conditions are preferably used to effect cleavage:

1) Excess of the URE over target DNA should be present to activate the enzyme. URE present only in equimolar amounts to the target DNA would yield poor cleavage of ssDNA because the amount of active enzyme available would be limiting.
2) An activator may be used to activate part of the *Fok*I enzyme to dimerize without causing cleavage. Examples of appropriate activators are shown in Table 14.
3) The cleavage reaction is performed at a temperature between 45°-75°C, preferably above 50°C and most preferably above 55°C.

The UREs used in the prior art contained a 14-base single-stranded segment, a 10-base stem (containing a *FokI* site), followed by the palindrome of the 10-base stem. While such UREs may be used in the methods of this invention, the preferred UREs of this invention also include a segment of three to eight bases (a loop) between the *FokI* restriction

endonuclease recognition site containing segments. In the preferred embodiment, the stem (containing the *FokI* site) and its palindrome are also longer than 10 bases. Preferably, they are 10-14 bases in length. Examples of these "lollipop" URE adapters are shown in Table 15.

One example of using a URE to cleave an single-stranded DNA involves the FR3 region of human heavy chain. Table 16 shows an analysis of 840 full-length mature human heavy chains with the URE recognition sequences shown. The vast majority (718/840=0.85) will be recognized with 2 or fewer mismatches using five UREs (VHS881-1.1, VHS881-1.2, VHS881-2.1, VHS881-4.1, and VHS881-9.1). Each has a 20-base adaptor sequence to complement the germline gene, a ten-base stem segment containing a *Fok*I site, a five base loop, and the reverse complement of the first stem segment. Annealing those adapters, alone or in combination, to single-stranded antisense heavy chain DNA and treating with *Fok*I in the presence of, *e.g.*, the activator FOKIact, will lead to cleavage of the antisense strand at the position indicated.

Another example of using a URE(s) to cleave a single-stranded DNA involves the FR1 region of the human Kappa light chains. Table 17 shows an analysis of 182 full-length human kappa chains for matching by the four 19-base probe sequences shown. Ninety-six percent of the sequences match one of the probes with 2 or fewer mismatches. The URE adapters shown in Table 17 are for cleavage of the sense strand of kappa chains. Thus, the adaptor sequences are the reverse complement of the germline gene sequences. The URE consists of a ten-base stem, a five base loop, the reverse complement of the stem and the complementation

sequence. The loop shown here is TTGTT, but other sequences could be used. Its function is to interrupt the palindrome of the stems so that formation of a lollypop monomer is favored over dimerization. Table 17 also shows where the sense strand is cleaved.

Another example of using a URE to cleave a single-stranded DNA involves the human lambda light chain. Table 18 shows analysis of 128 human lambda light chains for matching the four 19-base probes shown. With three or fewer mismatches, 88 of 128 (69%) of the chains match one of the probes. Table 18 also shows URE adapters corresponding to these probes. Annealing these adapters to upper-strand ssDNA of lambda chains and treatment with *Fok*I in the presence of FOKIact at a temperature at or above 45°C will lead to specific and precise cleavage of the chains.

The conditions under which the short oligonucleotide sequences of the first method and the UREs of the second method are contacted with the single-stranded DNAs may be empirically determined. The conditions must be such that the single-stranded DNA remains in substantially single-stranded form. More particularly, the conditions must be such that the single-stranded DNA does not form loops that may interfere with its association with the oligonucleotide sequence or the URE or that may themselves provide sites for cleavage by the chosen restriction endonuclease.

The effectiveness and specificity of short oligonucleotides (first method) and UREs (second method) can be adjusted by controlling the concentrations of the URE adapters/oligonucleotides and substrate DNA, the temperature, the pH, the concentration of metal ions, the ionic strength, the

concentration of chaotropes (such as urea and formamide), the concentration of the restriction endonuclease(*e.g.*, *Fok*I), and the time of the digestion. These conditions can be optimized with synthetic oligonucleotides having: 1) target germline gene sequences, 2) mutated target gene sequences, or 3) somewhat related non-target sequences. The goal is to cleave most of the target sequences and minimal amounts of non-targets.

In accordance with this invention, the single-stranded DNA is maintained in substantially that form using a temperature between about 37°C and about 75°C. Preferably, a temperature between about 45°C and about 75°C is used. More preferably, a temperature between 50°C and 60°C, most preferably between 55°C and 60°C, is used. These temperatures are employed both when contacting the DNA with the oligonucleotide or URE and when cleaving the DNA using the methods of this invention.

The two cleavage methods of this invention have several advantages. The first method allows the individual members of the family of single-stranded DNAs to be cleaved preferentially at one substantially conserved endonuclease recognition site. The method also does not require an endonuclease recognition site to be built into the reverse transcription or amplification primers. Any native or synthetic site in the family can be used.

The second method has both of these advantages. In addition, the preferred URE method allows the single-stranded DNAs to be cleaved at positions where no endonuclease recognition site naturally occurs or has been synthetically constructed.

Most importantly, both cleavage methods permit the use of 5' and 3' primers so as to maximize diversity and then cleavage to remove unwanted or deleterious sequences before cloning, display and/or expression.

After cleavage of the amplified DNAs using one of the methods of this invention, the DNA is prepared for cloning, display and/or expression. This is done by using a partially duplexed synthetic DNA adapter, whose terminal sequence is based on the specific cleavage site at which the amplified DNA has been cleaved.

The synthetic DNA is designed such that when it is ligated to the cleaved single-stranded DNA in proper reading frame so that the desired peptide, polypeptide or protein can be displayed on the surface of the genetic package and/or expressed. Preferably, the double-stranded portion of the adapter comprises the sequence of several codons that encode the amino acid sequence characteristic of the family of peptides, polypeptides or proteins up to the cleavage site. For human heavy chains, the amino acids of the 3-23 framework are preferably used to provide the sequences required for expression of the cleaved DNA.

Preferably, the double-stranded portion of the adapter is about 12 to 100 bases in length. More preferably, about 20 to 100 bases are used. The double-standard region of the adapter also preferably contains at least one endonuclease recognition site useful for cloning the DNA into a suitable display and/or expression vector (or a recipient vector used to archive the diversity). This endonuclease restriction site may be native to the germline gene sequences used to extend the DNA sequence. It may be also constructed

using degenerate sequences to the native germline gene sequences. Or, it may be wholly synthetic.

The single-stranded portion of the adapter is complementary to the region of the cleavage in the single-stranded DNA. The overlap can be from about 2 bases up to about 15 bases. The longer the overlap, the more efficient the ligation is likely to be. A preferred length for the overlap is 7 to 10. This allows some mismatches in the region so that diversity in this region may be captured.

The single-stranded region or overlap of the partially duplexed adapter is advantageous because it allows DNA cleaved at the chosen site, but not other fragments to be captured. Such fragments would contaminate the library with genes encoding sequences that will not fold into proper antibodies and are likely to be non-specifically sticky.

One illustration of the use of a partially duplexed adaptor in the methods of this invention involves ligating such adaptor to a human FR3 region that has been cleaved, as described above, at 5'-ACnGT-3' using HpyCH4III, Bst4CI or TaaI.

Table 4 F.2 shows the bottom strand of the double-stranded portion of the adaptor for ligation to the cleaved bottom-strand DNA. Since the HpyCH4III-Site is so far to the right (as shown in Table 3), a sequence that includes the *Afl*II-site as well as the *Xba*I site can be added. This bottom strand portion of the partially-duplexed adaptor, H43.XAExt, incorporates both *Xba*I and *Afl*II-sites. The top strand of the double-stranded portion of the adaptor has neither site (due to planned mismatches in the segments opposite the *Xba*I and *Afl*II-Sites of H43.XAExt), but

will anneal very tightly to H43.XAExt. H43AExt contains only the *Afl*II-site and is to be used with the top strands H43.ABr1 and H43.ABr2 (which have intentional alterations to destroy the *Afl*II-site).

After ligation, the desired, captured DNA can be PCR amplified again, if desired, using in the preferred embodiment a primer to the downstream constant region of the antibody gene and a primer to part of the double-standard region of the adapter. The primers may also carry restriction endonuclease sites for use in cloning the amplified DNA.

After ligation, and perhaps amplification, of the partially double-stranded adapter to the single-stranded amplified DNA, the composite DNA is cleaved at chosen 5' and 3' endonuclease recognition sites.

The cleavage sites useful for cloning depend on the phage or phagemid or other vectors into which the cassette will be inserted and the available sites in the antibody genes. Table 19 provides restriction endonuclease data for 75 human light chains. Table 20 shows corresponding data for 79 human heavy chains. In each Table, the endonucleases are ordered by increasing frequency of cutting. In these Tables, Nch is the number of chains cut by the enzyme and Ns is the number of sites (some chains have more than one site).

From this analysis, *Sfi*I, *Not*I, *Afl*II, *Apa*LI, and *Asc*I are very suitable. *Sfi*I and *Not*I are preferably used in pCES1 to insert the heavy-chain display segment. *Apa*LI and *Asc*I are preferably used in pCES1 to insert the light-chain display segment.

*Bst*EII-sites occur in 97% of germ-line JH genes. In rearranged V genes, only 54/79 (68%) of heavy-chain genes contain a *Bst*EII-Site and 7/61 of

these contain two sites. Thus, 47/79 (59%) contain a single *Bst*EII-Site. An alternative to using *Bst*EII is to cleave *via* UREs at the end of JH and ligate to a synthetic oligonucleotide that encodes part of CH1.

One example of preparing a family of DNA sequences using the methods of this invention involves capturing human CDR 3 diversity. As described above, mRNAs from various autoimmune patients are reverse transcribed into lower strand cDNA. After the top strand RNA is degraded, the lower strand is immobilized and a short oligonucleotide used to cleave the cDNA upstream of CDR3. A partially duplexed synthetic DNA adapter is then annealed to the DNA and the DNA is amplified using a primer to the adapter and a primer to the constant region (after FR4). The DNA is then cleaved using *BstEII* (in FR4) and a restriction endonuclease appropriate to the partially double-stranded adapter (*e.g.*, *XbaI* and *AflII* (in FR3)). The DNA is then ligated into a synthetic VH skeleton such as 3-23.

One example of preparing a single-stranded DNA that was cleaved using the URE method involves the human Kappa chain. The cleavage site in the sense strand of this chain is depicted in Table 17. The oligonucleotide kapextURE is annealed to the oligonucleotides (kaBR01UR, kaBR02UR, kaBR03UR, and kaBR04UR) to form a partially duplex DNA. This DNA is then ligated to the cleaved soluble kappa chains. The ligation product is then amplified using primers kapextUREPCR and CKForeAsc (which inserts a AscI site after the end of C kappa). This product is then cleaved with *Apa*LI and *Asc*I and ligated to similarly cut recipient vector.

Another example involves the cleavage of lambda light chains, illustrated in Table 18. After cleavage, an extender (ON_LamEx133) and four bridge oligonucleotides (ON_LamB1-133, ON_LamB2-133, ON_LamB3-133, and ON_LamB4-133) are annealed to form a partially duplex DNA. That DNA is ligated to the cleaved lambda-chain sense strands. After ligation, the DNA is amplified with ON_Lam133PCR and a forward primer specific to the lambda constant domain, such as CL2ForeAsc or CL7ForeAsc (Table 130).

In human heavy chains, one can cleave almost all genes in FR4 (downstream, *i.e.*, toward the 3' end of the sense strand, of CDR3) at a *Bst*EII-Site that occurs at a constant position in a very large fraction of human heavy-chain V genes. One then needs a site in FR3, if only CDR3 diversity is to be captured, in FR2, if CDR2 and CDR3 diversity is wanted, or in FR1, if all the CDR diversity is wanted. These sites are preferably inserted as part of the partially double-stranded adaptor.

The preferred process of this invention is to provide recipient vectors (*e.g.*, for display and/or expression) having sites that allow cloning of either light or heavy chains. Such vectors are well known and widely used in the art. A preferred phage display vector in accordance with this invention is phage MALIA3. This displays in gene III. The sequence of the phage MALIA3 is shown in Table 21A (annotated) and Table 21B (condensed).

The DNA encoding the selected regions of the light or heavy chains can be transferred to the vectors using endonucleases that cut either light or heavy chains only very rarely. For example, light chains may

be captured with *Apa*LI and *Asc*I. Heavy-chain genes are preferably cloned into a recipient vector having *Sfi*I, *Nco*I, *Xba*I, *Afl*II, *Bst*EII, *Apa*I, and *Not*I sites. The light chains are preferably moved into the library as *Apa*LI-*Asc*I fragments. The heavy chains are preferably moved into the library as *Sfi*I-*Not*I fragments.

Most preferably, the display is had on the surface of a derivative of M13 phage. The most preferred vector contains all the genes of M13, an antibiotic resistance gene, and the display cassette. The preferred vector is provided with restriction sites that allow introduction and excision of members of the diverse family of genes, as cassettes. The preferred vector is stable against rearrangement under the growth conditions used to amplify phage.

In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed in a phagemid vector (*e.g.*, pCES1) that displays and/or expresses the peptide, polypeptide or protein. Such vectors may also be used to store the diversity for subsequent display and/or expression using other vectors or phage.

In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed in a yeast vector.

In another embodiment, the mode of display may be through a short linker to anchor domains -- one possible anchor comprising the final portion of M13 III ("IIIstump") and a second possible anchor being the full length III mature protein.

The IIIstump fragment contains enough of M13

III to assemble into phage but not the domains involved in mediating infectivity. Because the w.t. III proteins are present the phage is unlikely to delete the antibody genes and phage that do delete these segments receive only a very small growth advantage. For each of the anchor domains, the DNA encodes the w.t. AA sequence, but differs from the w.t. DNA sequence to a very high extent. This will greatly reduce the potential for homologous recombination between the anchor and the w.t. gene that is also present (see Example 6).

Most preferably, the present invention uses a complete phage carrying an antibiotic-resistance gene (such as an ampicillin-resistance gene) and the display cassette. Because the w.t. *iii* and possibly *viii* genes are present, the w.t. proteins are also present. The display cassette is transcribed from a regulatable promoter (*e.g.*, P_{Lacz}). Use of a regulatable promoter allows control of the ratio of the fusion display gene to the corresponding w.t. coat protein. This ratio determines the average number of copies of the display fusion per phage (or phagemid) particle.

Another aspect of the invention is a method of displaying peptides, polypeptides or proteins (and particularly Fabs) on filamentous phage. In the most preferred embodiment this method displays FABs and comprises:

a) obtaining a cassette capturing a diversity of segments of DNA encoding the elements:

P_{reg}::RBS1::SS1::VL::CL::stop::RBS2::SS2::VH::CH1::linker::anchor::stop::,

where P_{reg} is a regulatable promoter, RBS1 is a first

ribosome binding site, SS1 is a signal sequence operable in the host strain, VL is a member of a diverse set of light-chain variable regions, CL is a light-chain constant region, stop is one or more stop codons, RBS2 is a second ribosome binding site, SS2 is a second signal sequence operable in the host strain, VH is a member of a diverse set of heavy-chain variable regions, CH1 is an antibody heavy-chain first constant domain, linker is a sequence of amino acids of one to about 50 residues, anchor is a protein that will assemble into the filamentous phage particle and stop is a second example of one or more stop codons; and

b) positioning that cassette within the phage genome to maximize the viability of the phage and to minimize the potential for deletion of the cassette or parts thereof.

The DNA encoding the anchor protein in the above preferred cassette should be designed to encode the same (or a closely related) amino acid sequence as is found in one of the coat proteins of the phage, but with a distinct DNA sequence. This is to prevent unwanted homologous recombination with the w.t. gene. In addition, the cassette should be placed in the intergenic region. The positioning and orientation of the display cassette can influence the behavior of the phage.

In one embodiment of the invention, a transcription terminator may be placed after the second stop of the display cassette above (*e.g.*, Trp). This will reduce interaction between the display cassette and other genes in the phage antibody display vector.

In another embodiment of the methods of this invention, the phage or phagemid can display and/or

express proteins other than Fab, by replacing the Fab portions indicated above, with other protein genes.

Various hosts can be used the display and/or expression aspect of this invention. Such hosts are well known in the art. In the preferred embodiment, where Fabs are being displayed and/or expressed, the preferred host should grow at 30°C and be $RecA^-$ (to reduce unwanted genetic recombination) and $EndA^-$ (to make recovery of RF DNA easier). It is also preferred that the host strain be easily transformed by electroporation.

XL1-Blue MRF' satisfies most of these preferences, but does not grow well at 30°C. XL1-Blue MRF' does grow slowly at 38°C and thus is an acceptable host. TG-1 is also an acceptable host although it is $RecA^+$ and $EndA^+$. XL1-Blue MRF' is more preferred for the intermediate host used to accumulate diversity prior to final construction of the library.

After display and/or expression, the libraries of this invention may be screened using well known and conventionally used techniques. The selected peptides, polypeptides or proteins may then be used to treat disease. Generally, the peptides, polypeptides or proteins for use in therapy or in pharmaceutical compositions are produced by isolating the DNA encoding the desired peptide, polypeptide or protein from the member of the library selected. That DNA is then used in conventional methods to produce the peptide, polypeptides or protein it encodes in appropriate host cells, preferably mammalian host cells, *e.g.*, CHO cells. After isolation, the peptide, polypeptide or protein is used alone or with pharmaceutically acceptable compositions in therapy to treat disease.

EXAMPLES

Example 1: RACE amplification of heavy and light chain antibody repertoires from autoimmune patients.

Total RNA was isolated from individual blood samples (50 ml) of 11 patients using a RNAzolTM kit (CINNA/Biotecx), as described by the manufacturer. The patients were diagnosed as follows:

1. SLE and phospholipid syndrome
2. limited systemic sclerosis
3. SLE and Sjogren syndrome
4. Limited Systemic sclerosis
5. Reumatoid Arthritis with active vasculitis
6. Limited systemic sclerosis and Sjogren Syndrome
7. Reumatoid Artritis and (not active) vasculitis
8. SLE and Sjogren syndrome
9. SLE
10. SLE and (active) glomerulonephritis
11. Polyarthritis/ Raynauds Phenomen

From these 11 samples of total RNA, Poly-A+ RNA was isolated using Promega PolyATtract® mRNA Isolation kit (Promega).

250 ng of each poly-A+ RNA sample was used to amplify antibody heavy and light chains with the GeneRAacerTM kit (Invitrogen cat no. L1500-01). A schematic overview of the RACE procedure is shown in FIG. 3.

Using the general protocol of the GeneRAacer™ kit, an RNA adaptor was ligated to the 5'end of all mRNAs. Next, a reverse transcriptase reaction was performed in the presence of oligo(dT15) specific

primer under conditions described by the manufacturer in the GeneRAacer™ kit.

1/5 of the cDNA from the reverse transcriptase reaction was used in a 20 ul PCR reaction. For amplification of the heavy chain IgM repertoire, a forward primer based on the CH1 chain of IgM [HuCmFOR] and a backward primer based on the ligated synthetic adaptor sequence [5'A] were used. (See Table 22)

For amplification of the kappa and lambda light chains, a forward primer that contains the 3' coding-end of the cDNA [HuCkFor and HuCLFor2+HuCLfor7] and a backward primer based on the ligated synthetic adapter sequence [5'A] was used (See Table 22). Specific amplification products after 30 cycles of primary PCR were obtained.

FIG. 4 shows the amplification products obtained after the primary PCR reaction from 4 different patient samples. 8 ul primary PCR product from 4 different patients was analyzed on a agarose gel [labeled 1,2, 3 and 4]. For the heavy chain, a product of approximately 950 nt is obtained while for the kappa and lambda light chains the product is approximately 850 nt. M1-2 are molecular weight markers.

PCR products were also analyzed by DNA sequencing [10 clones from the lambda, kappa or heavy chain repertoires]. All sequenced antibody genes recovered contained the full coding sequence as well as the 5' leader sequence and the V gene diversity was the expected diversity (compared to literature data).

50 ng of all samples from all 11 individual amplified samples were mixed for heavy, lambda light or kappa light chains and used in secondary PCR reactions.

In all secondary PCRs approximately 1 ng

template DNA from the primary PCR mixture was used in multiple 50 ul PCR reactions [25 cycles].

For the heavy chain, a nested biotinylated forward primer [HuCm-Nested] was used, and a nested 5'end backward primer located in the synthetic adapter-sequence [5'NA] was used. The 5'end lower-strand of the heavy chain was biotinylated.

For the light chains, a 5'end biotinylated nested primer in the synthetic adapter was used [5'NA] in combination with a 3'end primer in the constant region of Ckappa and Clambda, extended with a sequence coding for the AscI restriction site [kappa: HuCkForAscI, Lambda: HuCL2-FOR-ASC + HuCL7-FOR-ASC]. [5'end Top strand DNA was biotinylated]. After gel-analysis the secondary PCR products were pooled and purified with Promega Wizzard PCR cleanup. Approximately 25 ug biotinylated heavy chain, lambda and kappa light chain DNA was isolated from the 11 patients.

Example 2: Capturing kappa chains with BsmAI.

A repertoire of human-kappa chain mRNAs was prepared using the RACE method of Example 1 from a collection of patients having various autoimmune diseases.

This Example followed the protocol of Example 1. Approximately 2 micrograms (ug) of human kappa-chain (Igkappa) gene RACE material with biotin attached to 5'-end of upper strand was immobilized as in Example 1 on 200 microliters (µL) of Seradyn magnetic beads. The lower strand was removed by washing the DNA with 2 aliquots 200 µL of 0.1 M NaOH (pH 13) for 3 minutes for the first aliquot followed by 30 seconds for the second

aliquot. The beads were neutralized with 200 µL of 10 mM Tris (pH 7.5) 100 mM NaCl. The short oligonucleotides shown in Table 23 were added in 40 fold molar excess in 100 µL of NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol pH 7.9) to the dry beads. The mixture was incubated at 95°C for 5 minutes then cooled down to 55°C over 30 minutes. Excess oligonucleotide was washed away with 2 washes of NEB buffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol pH 7.9). Ten units of BsmAI (NEB) were added in NEB buffer 3 and incubated for 1 h at 55°C. The cleaved downstream DNA was collected and purified over a Qiagen PCR purification column (FIGs. 5 and 6).

FIG. 5 shows an analysis of digested kappa single-stranded DNA. Approximately 151.5 pmol of adapter was annealed to 3.79 pmol of immobilized kappa single-stranded DNA followed by digestion with 15 U of *BsmAI*. The supernatant containing the desired DNA was removed and analyzed by 5% polyacrylamide gel along with the remaining beads which contained uncleaved full length kappa DNA. 189 pmol of cleaved single-stranded DNA was purified for further analysis. Five percent of the original full length ssDNA remained on the beads.

FIG. 6 shows an analysis of the extender - cleaved kappa ligation. 180 pmol of pre-annealed bridge/extender was ligated to 1.8 pmol of *BsmAI* digested single-stranded DNA. The ligated DNA was purified by Qiagen PCR purification column and analyzed on a 5% polyacrylamide gel. Results indicated that the ligation of extender to single-stranded DNA was 95% efficient.

A partially double-stranded adaptor was prepared using the oligonucleotide shown in Table 23.

The adaptor was added to the single-stranded DNA in 100 fold molar excess along with 1000 units of T4 DNA ligase and incubated overnight at 16°C. The excess oligonucleotide was removed with a Qiagen PCR purification column. The ligated material was amplified by PCR using the primers kapPCRt1 and kapfor shown in Table 23 for 10 cycles with the program shown in Table 24.

The soluble PCR product was run on a gel and showed a band of approximately 700 n, as expected (FIGs. 7 and 8). The DNA was cleaved with enzymes *Apa*LI and *Asc*I, gel purified, and ligated to similarly cleaved vector pCES1.

FIG. 7 shows an analysis of the PCR product from the extender-kappa amplification. Ligated extender-kappa single-stranded DNA was amplified with primers specific to the extender and to the constant region of the light chain. Two different template concentrations, 10 ng versus 50 ng, were used as template and 13 cycles were used to generate approximately 1.5 ug of dsDNA as shown by 0.8% agarose gel analysis.

FIG. 8 shows an analysis of the purified PCR product from the extender-kappa amplification. Approximately 5 ug of PCR amplified extender-kappa double-stranded DNA was run out on a 0.8% agarose gel, cut out, and extracted with a GFX gel purification column. By gel analysis, 3.5 ug of double-stranded DNA was prepared.

The assay for capturing kappa chains with BsmA1 was repeated and produced similar results. FIG 9A shows the DNA after it was cleaved and collected and purified over a Qiagen PCR purification column. FIG. 9B shows the partially double-stranded adaptor

ligated to the single-stranded DNA. This ligated material was then amplified (FIG. 9C). The gel showed a band of approximately 700 n.

Table 25 shows the DNA sequence of a kappa light chain captured by this procedure. Table 26 shows a second sequence captured by this procedure. The closest bridge sequence was complementary to the sequence 5'-agccacc-3', but the sequence captured reads 5'-Tgccacc-3', showing that some mismatch in the overlapped region is tolerated.

Example 3: Construction of Synthetic CDR1 and CDR2 Diversity in V-3-23 VH Framework.

Synthetic diversity in Complementary Determinant Region (CDR) 1 and 2 was created in the 3-23 VH framework in a two step process: first, a vector containing the 3-23 VH framework was constructed; and then, a synthetic CDR 1 and 2 was assembled and cloned into this vector.

For construction of the 3-23 VH framework, 8 oligonucleotides and two PCR primers (long oligonucleotides - TOPFR1A, BOTFR1B, BOTFR2, BOTFR3, F06, BOTFR4, ON-vgC1, and ON-vgC2 and primers - SFPRMET and BOTPCRPRIM, shown in Table 27) that overlap were designed based on the Genebank sequence of 3-23 VH framework region. The design incorporated at least one useful restriction site in each framework region, as shown in Table 27. In Table 27, the segments that were synthesized are shown as bold, the overlapping regions are underscored, and the PCR priming regions at each end are underscored.

A mixture of these 8 oligos was combined at a final concentration of 2.5uM in a 20ul PCR reaction. The PCR mixture contained 200uM dNTPs, 2.5mM $MgCl_2$, 0.02U *Pfu Turbo*™ DNA Polymerase, 1U Qiagen HotStart Taq DNA Polymerase, and 1X Qiagen PCR buffer. The PCR program consisted of 10 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 30s.

The assembled 3-23 VH DNA sequence was then amplified, using 2.5ul of a 10-fold dilution from the initial PCR in 100ul PCR reaction. The PCR reaction contained 200uM dNTPs, 2.5mM $MgCl_2$, 0.02U *Pfu Turbo*™ DNA Polymerase, 1U Qiagen HotStart Taq DNA Polymerase, 1X Qiagen PCR Buffer and 2 outside primers (SFPRMET and BOTPCRPRIM) at a concentration of 1uM. The PCR program consisted of 23 cycles at 94°C for 30s, 55°C for 30s, and 72°C for 60s. The 3-23 VH DNA sequence was digested and cloned into pCES1 (phagemid vector) using the *Sfi*I and *Bst*EII restriction endonuclease sites. All restriction enzymes mentioned herein were supplied by New England BioLabs, Beverly, MA and used as per the manufacturer's instructions.

Stuffer sequences (shown in Table 28 and Table 29) were introduced into pCES1 to replace CDR1/CDR2 sequences (900 bases between *Bsp*EI and *Xba*I RE sites) and CDR3 sequences (358 bases between *Afl*II and *Bst*EII) prior to cloning the CDR1/CDR2 diversity. This new vector was termed pCES5 and its sequence is given in Table 29.

Having stuffers in place of the CDRs avoids the risk that a parental sequence would be over-represented in the library. The stuffer sequences are fragments from the penicillase gene of *E. coli*. The CDR1-2 stuffer contains restriction sites for *Bgl*II,

*Bsu*36I, *Bcl*I, *Xcm*I, *Mlu*I, *Pvu*II, *Hpa*I, and *Hinc*II, the underscored sites being unique within the vector pCES5. The stuffer that replaces CDR3 contains the unique restriction endonuclease site *Rsr*II.

A schematic representation of the design for CDR1 and CDR2 synthetic diversity is shown FIG. 10. The design was based on the presence of mutations in DP47/3-23 and related germline genes. Diversity was designed to be introduced at the positions within CDR1 and CDR2 indicated by the numbers in FIG. 10. The diversity at each position was chosen to be one of the three following schemes: 1 = ADEFGHIKLMNPQRSTVWY; 2 = YRWVGS; 3 = PS, in which letters encode equimolar mixes of the indicated amino acids.

For the construction of the CDR1 and CDR2 diversity, 4 overlapping oligonucleotides (ON-vgC1, ON_Br12, ON_CD2Xba, and ON-vgC2, shown in Table 27 and Table 30) encoding CDR1/2, plus flanking regions, were designed. A mixture of these 4 oligos was combined at a final concentration of 2.5uM in a 40ul PCR reaction. Two of the 4 oligos contained variegated sequences positioned at the CDR1 and the CDR2. The PCR mixture contained 200uM dNTPs, 2.5U Pwo DNA Polymerase (Roche), and 1X Pwo PCR buffer with 2mM $MgSO_4$. The PCR program consisted of 10 cycles at 94°C for 30s, 60°C for 30s, and 72°C for 60s. This assembled CDR1/2 DNA sequence was amplified, using 2.5ul of the mixture in 100ul PCR reaction. The PCR reaction contained 200uM dNTPs, 2.5U Pwo DNA Polymerase, 1X Pwo PCR Buffer with 2mM $MgSO_4$ and 2 outside primers at a concentration of 1uM. The PCR program consisted of 10 cycles at 94°C for 30s, 60°C for 30s, and 72°C for 60s. These variegated sequences were digested and cloned into the 3-23 VH framework in place of the CDR1/2 stuffer.

We obtained approximately 7 X 10^7 independent transformants. CDR3 diversity either from donor populations or from synthetic DNA can be cloned into the vector containing synthetic CDR1 and CDR 2 diversity.

A schematic representation of this procedure is shown in FIG. 11. A sequence encoding the FR-regions of the human V3-23 gene segment and CDR regions with synthetic diversity was made by oligonucleotide assembly and cloning via *BspE1* and *Xbal* sites into a vector that complements the FR1 and FR3 regions. Into this library of synthetic VH segments, the complementary VH-CDR3 sequence (top right) was cloned via Xbal an BstEll sites. The resulting cloned CH genes contain a combination of designed synthetic diversity and natural diversity (see FIG. 11).

Example 4: Cleavage and ligation of the lambda light chains with HinfI.

A schematic of the cleavage and ligation of antibody light chains is shown in FIGs. 12A and 12B. Approximately 2 ug of biotinylated human Lambda DNA prepared as described in Example 1 was immobilized on 200 ul Seradyn magnetic beads. The lower strand was removed by incubation of the DNA with 200 ul of 0.1 M NaOH (pH=13) for 3 minutes, the supernatant was removed and an additional washing of 30 seconds with 200 ul of 0.1 M NaOH was performed. Supernatant was removed and the beads were neutralized with 200 ul of 10 mM Tris (pH=7.5), 100 mM NaCl. 2 additional washes with 200 ul NEB2 buffer 2, containing 10 mM Tris (pH=7.9), 50 mM NaCl, 10 mM MgCl2 and 1 mM dithiothreitol, were

performed. After immobilization, the amount of ssDNA was estimated on a 5% PAGE-UREA gel.

About 0.8 ug ssDNA was recovered and incubated in 100 ul NEB2 buffer 2 containing 80 molar fold excess of an equimolar mix of ON_Lam1aB7, ON_Lam2aB7, ON_Lam3lB7 and ON_Lam3rB7 [each oligo in 20 fold molar excess] (see Table 31).

The mixture was incubated at 95° C for 5 minutes and then slowly cooled down to 50° C over a period of 30 minutes. Excess of oligonucleotide was washed away with 2 washes of 200 ul of NEB buffer 2. 4 U/ug of *Hinf I* was added and incubated for 1 hour at 50° C. Beads were mixed every 10 minutes.

After incubation the sample was purified over a Qiagen PCR purification column and was subsequently analysed on a 5% PAGE-urea gel (see FIG. 13A, cleavage was more than 70% efficient).

A schematic of the ligation of the cleaved light chains is shown in FIG. 12B. A mix of bridge/extender pairs was prepared from the Brg/Ext oligo's listed in Table 31 (total molar excess 100 fold) in 1000 U of T4 DNA Ligase (NEB) and incubated overnight at 16° C. After ligation of the DNA, the excess oligonucleotide was removed with a Qiagen PCR purification column and ligation was checked on a Urea-PAGE gel (see FIG. 13B; ligation was more than 95% efficient).

Multiple PCRs were performed containing 10 ng of the ligated material in an 50 ul PCR reaction using 25 pMol ON lamPlePCR and 25 pmol of an equimolar mix of Hu-CL2AscI/HuCL7AscI primer (see Example 1).

PCR was performed at 60° C for 15 cycles using Pfu polymerase. About 1 ug of dsDNA was recovered

per PCR (see FIG. 13C) and cleaved with *ApaL1* and *AscI* for cloning the lambda light chains in pCES2.

Example 5: Capture of human heavy-chain CDR3 population.

A schematic of the cleavage and ligation of antibody light chains is shown in FIGs. 14A and 14B.

Approximately 3 ug of human heavy-chain (IgM) gene RACE material with biotin attached to 5'-end of lower strand was immobilized on 300 uL of Seradyn magnetic beads. The upper strand was removed by washing the DNA with 2 aliquots 300 uL of 0.1 M NaOH (pH 13) for 3 minutes for the first aliquot followed by 30 seconds for the second aliquot. The beads were neutralized with 300 uL of 10 mM Tris (pH 7.5) 100 mM NaCl. The REdaptors (oligonucleotides used to make single-stranded DNA locally double-stranded) shown in Table 32 were added in 30 fold molar excess in 200 uL of NEB buffer 4 (50 mM Potasium Acetate, 20 mM Tris-Acetate, 10 mM Magnesuim Acetate, 1 mM dithiothreitol pH 7.9) to the dry beads. The REadaptors were incubated with the single-stranded DNA at 80 °C for 5 minutes then cooled down to 55 °C over 30 minutes. Excess REdaptors were washed away with 2 washes of NEB buffer 4. Fifteen units of HpyCH4III (NEB) were added in NEB buffer 4 and incubated for 1 hour at 55 °C. The cleaved downstream DNA remaining on the beads was removed from the beads using a Qiagen Nucleotide removal column (see FIG. 15).

The Bridge/Extender pairs shown in Table 33 were added in 25 molar excess along with 1200 units of T4 DNA ligase and incubated overnight at 16 °C. Excess

Bridge/Extender was removed with a Qiagen PCR purification column. The ligated material was amplified by PCR using primers H43.XAExtPCR2 and Hucumnest shown in Table 34 for 10 cycles with the program shown in Table 35.

The soluble PCR product was run on a gel and showed a band of approximately 500 n, as expected (see FIG. 15B). The DNA was cleaved with enzymes *SfiI* and *NotI*, gel purified, and ligated to similarly cleaved vector PCES1.

Example 6: Description of Phage Display Vector CJRA05, a member of the library built in vector DY3F7.

Table 36 contains an annotated DNA sequence of a member of the library, CJRA05, see FIG. 16. Table 36 is to be read as follows: on each line everything that follows an exclamation mark "!" is a comment. All occurrences of A, C, G, and T before "!" are the DNA sequence. Case is used only to show that certain bases constitute special features, such as restriction sites, ribosome binding sites, and the like, which are labeled below the DNA. CJRA05 is a derivative of phage DY3F7, obtained by cloning an *ApaLI* to *NotI* fragment into these sites in DY3F31. DY3F31 is like DY3F7 except that the light chain and heavy chain genes have been replaced by "stuffer" DNA that does not code for any antibody. DY3F7 contains an antibody that binds streptavidin, but did not come from the present library.

The phage genes start with gene ii and continue with genes x, v, vii, ix, viii, iii, vi, i, and iv. Gene iii has been slightly modified in that

eight codons have been inserted between the signal sequence and the mature protein and the final amino acids of the signal sequence have been altered. This allows restriction enzyme recognition sites *EagI* and *XbaI* to be present. Following gene iv is the phage origin of replication (ori). After ori is bla which confers resistance to ampicillin (ApR). The phage genes and bla are transcribed in the same sense.

After bla, is the Fab cassette (illustrated in FIG. 17) comprising:

a) PlacZ promoter,
b) A first Ribosome Binding Site (RBS1),
c) The signal sequence form M13 iii,
d) An *ApaLI* RERS,
e) A light chain (a kappa L20::JK1 shortened by one codon at the V-J boundary in this case),
f) An *AscI* RERS,
g) A second Ribosome Binding Site (RBS2),
h) A signal sequence, preferably PelB, which contains,
i) An *SfiI* RERS,
j) A synthetic 3-23 V region with diversity in CDR1 and CDR2,
k) A captured CDR3,
l) A partially synthetic J region (FR4 after BstEII),
m) CH1,
n) A NotI RERS,
o) A His6 tag,
p) A cMyc tag,
q) An amber codon,
r) An anchor DNA that encodes the same amino-acid sequence as codons 273 to 424 of M13 iii (as shown in Table 37).

s) Two stop codons,

t) An *AvrII* RERS, and

u) A trp terminator.

The anchor (item r) encodes the same amino-acid sequence as do codons 273 to 424 of M13 iii but the DNA is approximately as different as possible from the wild-type DNA sequence. In Table 36, the III' stump runs from base 8997 to base 9455. Below the DNA, as comments, are the differences with wild-type iii for the comparable codons with "!W.T" at the ends of these lines. Note that Met and Trp have only a single codon and must be left as is. These AA types are rare. Ser codons can be changed at all three base, while Leu and Arg codons can be changed at two.

In most cases, one base change can be introduced per codon. This has three advantages: 1) recombination with the wild-type gene carried elsewhere on the phage is less likely, 2) new restriction sites can be introduced, facilitating construction; and 3) sequencing primers that bind in only one of the two regions can be designed.

The fragment of M13 III shown in CJRA05 is the preferred length for the anchor segment. Alternative longer or shorter anchor segments defined by reference to whole mature III protein may also be utilized.

The sequence of M13 III consists of the following elements: Signal Sequence::Domain 1 (D1)::Linker 1 (L1)::Domain 2 (D2)::Linker 2 (L2)::Domain 3 (D3)::Transmembrane Segment (TM):: Intracellular anchor (IC) (see Table 38).

The pIII anchor (also known as trpIII) preferably consists of D2::L2::D3::TM::IC. Another embodiment for the pIII anchor consists of

D2'::L2::D3::TM::IC (where D2' comprises the last 21 residues of D2 with the first 109 residues deleted). A further embodiment of the pIII anchor consists of D2'(C>S)::L2::D3::TM::IC (where D2'(C>S) is D2' with the single C converted to S), and d) D3::TM::IC.

Table 38 shows a gene fragment comprising the *NotI* site, His6 tag, cMyc tag, an amber codon, a recombinant enterokinase cleavage site, and the whole of mature M13 III protein. The DNA used to encode this sequence is intentionally very different from the DNA of wild-type gene iii as shown by the lines denoted "W.T." containing the w.t. bases where these differ from this gene. III is divided into domains denoted "domain 1", "linker 1", "domain 2", "linker 2", "domain 3", "transmembrane segment", and "intracellular anchor".

Alternative preferred anchor segments (defined by reference to the sequence of Table 38) include:

codons 1-29 joined to codons 104-435, deleting domain 1 and retaining linker 1 to the end;

codons 1-38 joined to codons 104-435, deleting domain 1and retaining the rEK cleavage site plus linker 1 to the end from III;

codons 1-29 joined to codons 236-435, deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end;

codons 1-38 joined to codons 236-435, deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end and the rEK cleavage site;

codons 1-29 joined to codons 236-435 and changing codon 240 to Ser(*e.g.*, agc), deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end; and

codons 1-38 joined to codons 236-435 and changing codon 240 to Ser(*e.g.*, agc), deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end and the rEK cleavage site.

The constructs would most readily be made by methods similar to those of Wang and Wilkinson (Biotechniques 2001: 31(4)722-724) in which PCR is used to copy the vector except the part to be deleted and matching restriction sites are introduced or retained at either end of the part to be kept. Table 39 shows the oligonucleotides to be used in deleting parts of the III anchor segment. The DNA shown in Table 38 has an *NheI* site before the DINDDRMA recombinant enterokinase cleavage site (rEKCS). If *NheI* is used in the deletion process with this DNA, the rEKCS site would be lost. This site could be quite useful in cleaving Fabs from the phage and might facilitate capture of very high-afffinity antibodies. One could mutagenize this sequence so that the *NheI* site would follow the rEKCS site, an Ala Ser amino-acid sequence is already present. Alternatively, one could use *SphI* for the deletions. This would involve a slight change in amino acid sequence but would be of no consequence.

Example 7 : Selection of antigen binders from an enriched library of human antibodies using phage vector DY3F31.

In this example the human antibody library used is described in de Haard et al., (Journal of Biological Chemistry, 274 (26): 18218-30 (1999). This library, consisting of a large non-immune human Fab phagemid library, was first enriched on antigen, either

on streptavidin or on phenyl-oxazolone (phOx). The methods for this are well known in the art. Two preselected Fab libraries, the first one selected once on immobilized phOx-BSA (R1-ox) and the second one selected twice on streptavidin (R2-strep), were chosen for recloning.

These enriched repertoires of phage antibodies, in which only a very low percentage have binding activity to the antigen used in selection, were confirmed by screening clones in an ELISA for antigen binding. The selected Fab genes were transferred from the phagemid vector of this library to the DY3F31 vector via *ApaL1-Not1* restriction sites.

DNA from the DY3F31 phage vector was pretreated with ATP dependent DNAse to remove chromosomal DNA and then digested with *ApaL1* and *Not1*. An extra digestion with *AscI* was performed in between to prevent self-ligation of the vector. The *ApaL1/NotI* Fab fragment from the preselected libraries was subsequently ligated to the vector DNA and transformed into competent XL1-blue MRF' cells.

Libraries were made using vector:insert ratios of 1:2 for phOx-library and 1:3 for STREP library, and using 100 ng ligated DNA per 50 µl of electroporation-competent cells (electroporation conditions : one shock of 1700 V, 1 hour recovery of cells in rich SOC medium, plating on amplicillin-containing agar plates).

This transformation resulted in a library size of 1.6×10^6 for R1-ox in DY3F31 and 2.1×10^6 for R2-strep in DY3F31. Sixteen colonies from each library were screened for insert, and all showed the correct size insert (±1400 bp) (for both libraries).

Phage was prepared from these Fab libraries as follows. A representative sample of the library was inoculated in medium with ampicillin and glucose, and at OD 0.5, the medium exchanged for ampicillin and 1 mM IPTG. After overnight growth at 37 °C, phage was harvested from the supernatant by PEG-NaCl precipitation. Phage was used for selection on antigen. R1-ox was selected on phOx-BSA coated by passive adsorption onto immunotubes and R2-strep on streptavidin coated paramagnetic beads (Dynal, Norway), in procedures described in de Haard et. al. and Marks et. al., Journal of Molecular Biology, 222(3): 581-97 (1991). Phage titers and enrichments are given in Table 40.

Clones from these selected libraries, dubbed R2-ox and R3-strep respectively, were screened for binding to their antigens in ELISA. 44 clones from each selection were picked randomly and screened as phage or soluble Fab for binding in ELISA. For the libraries in DY3F31, clones were first grown in 2TY-2% glucose-50 µg/ml AMP to an OD600 of approximately 0.5, and then grown overnight in 2TY-50 µg/ml AMP +/- 1mM IPTG. Induction with IPTG may result in the production of both phage-Fab and soluble Fab. Therefore the (same) clones were also grown without IPTG. Table 41 shows the results of an ELISA screening of the resulting supernatant, either for the detection of phage particles with antigen binding (Anti-M13 HRP = anti-phage antibody), or for the detection of human Fabs, be it on phage or as soluble fragments, either with using the anti-myc antibody 9E10 which detects the myc-tag that every Fab carries at the C-terminal end of the heavy chain followed by a HRP-labeled rabbit-anti-Mouse serum (column 9E10/RAM-HRP), or with

anti-light chain reagent followed by a HRP-labeled goat-anti-rabbit antiserum(anti-CK/CL Gar-HRP).

The results shows that in both cases antigen-binders are identified in the library, with as Fabs on phage or with the anti-Fab reagents (Table 41). IPTG induction yields an increase in the number of positives. Also it can be seen that for the phOx-clones, the phage ELISA yields more positives than the soluble Fab ELISA, most likely due to the avid binding of phage. Twenty four of the ELISA-positive clones were screened using PCR of the Fab-insert from the vector, followed by digestion with *BstNI*. This yielded 17 different patterns for the phOx-binding Fab's in 23 samples that were correctly analyzed, and 6 out of 24 for the streptavidin binding clones. Thus, the data from the selection and screening from this pre-enriched non-immune Fab library show that the DY3F31 vector is suitable for display and selection of Fab fragments, and provides both soluble Fab and Fab on phage for screening experiments after selection.

Example 8: Selection of Phage-antibody libraries on streptavidin magnetic beads.

The following example describes a selection in which one first depletes a sample of the library of binders to streptavidin and optionally of binders to a non-target (*i.e.*, a molecule other than the target that one does not want the selected Fab to bind). It is hypothesized that one has a molecule, termed a "competitive ligand", which binds the target and that an antibody which binds at the same site would be especially useful.

For this procedure Streptavidin Magnetic Beads (Dynal) were blocked once with blocking solution (2% Marvel Milk, PBS (pH 7.4), 0.01% Tween-20 ("2%MPBST")) for 60 minutes at room temperature and then washed five times with 2%MPBST. 450 µL of beads were blocked for each depletion and subsequent selection set.

Per selection, 6.25 µL of biotinylated depletion target (1 mg/mL stock in PBST) was added to 0.250 mL of washed, blocked beads (from step 1). The target was allowed to bind overnight, with tumbling, at 4°C. The next day, the beads are washed 5 times with PBST.

Per selection, 0.010 mL of biotinylated target antigen (1 mg/mL stock in PBST) was added to 0.100 mL of blocked and washed beads (from step 1). The antigen was allowed to bind overnight, with tumbling, at 4°C. The next day, the beads were washed 5 times with PBST.

In round 1, 2 X 10^{12} up to 10^{13} plaque forming units (pfu) per selection were blocked against non-specific binding by adding to 0.500 mL of 2%MPBS (=2%MPBST without Tween) for 1 hr at RT (tumble). In later rounds, 1011 pfu per selection were blocked as done in round 1.

Each phage pool was incubated with 50 µL of depletion target beads (final wash supernatant removed just before use) on a Labquake rotator for 10 min at room temperature. After incubation, the phage supernatant was removed and incubated with another 50 µL of depletion target beads. This was repeated 3 more times using depletion target beads and twice using blocked streptavidin beads for a total of 7 rounds of

depletion, so each phage pool required 350 µL of depletion beads.

A small sample of each depleted library pool was taken for titering. Each library pool was added to 0.100 mL of target beads (final wash supernatant was removed just before use) and allowed to incubate for 2 hours at room temperature (tumble).

Beads were then washed as rapidly as possible (*e.g.*,3 minutes total) with 5 X 0.500 mL PBST and then 2X with PBS. Phage still bound to beads after the washing were eluted once with 0.250 mL of competitive ligand (~1 µµM) in PBST for 1 hour at room temperature on a Labquake rotator. The eluate was removed, mixed with 0.500 mL Minimal A salts solution and saved. For a second selection, 0.500 mL 100 mM TEA was used for elution for 10 min at RT, then neutralized in a mix of 0.250 mL of 1 M Tris, pH 7.4 + 0.500 mL Min A salts.

After the first selection elution, the beads can be eluted again with 0.300 mL of non-biotinylated target (1 mg/mL) for 1 hr at RT on a Labquake rotator. Eluted phage are added to 0.450 mL Minimal A salts.

Three eluates (competitor from 1st selection, target from 1st selection and neutralized TEA elution from 2nd selection) were kept separate and a small aliquot taken from each for titering. 0.500 mL Minimal A salts were added to the remaining bead aliquots after competitor and target elution and after TEA elution. Take a small aliquot from each was taken for tittering.

Each elution and each set of eluted beads was mixed with 2X YT and an aliquot (*e.g.*, 1 mL with 1. E 10/mL) of XL1-Blue MRF' E. coli cells (or other F' cell line) which had been chilled on ice after having been grown to mid-logarithmic phase, starved and

concentrated (see procedure below - "Mid-Log prep of XL-1 blue MRF' cells for infection").

After approximately 30 minutes at room temperature, the phage/cell mixtures were spread onto Bio-Assay Dishes (243 X 243 X 18 mm, Nalge Nunc) containing 2XYT, 1mM IPTG agar. The plates were incubated overnight at 30°C. The next day, each amplified phage culture was harvested from its respective plate. The plate was flooded with 35 mL TBS or LB, and cells were scraped from the plate. The resuspended cells were transferred to a centrifuge bottle. An additional 20 mL TBS or LB was used to remove any cells from the plate and pooled with the cells in the centrifuge bottle. The cells were centrifuged out, and phage in the supernatant was recovered by PEG precipitation. Over the next day, the amplified phage preps were titered.

In the first round, two selections yielded five amplified eluates. These amplified eluates were panned for 2-3 more additional rounds of selection using ~1. E 12 input phage/round. For each additional round, the depletion and target beads were prepared the night before the round was initiated.

For the elution steps in subsequent rounds, all elutions up to the elution step from which the amplified elution came from were done, and the previous elutions were treated as washes. For the bead infection amplified phage, for example, the competitive ligand and target elutions were done and then tossed as washes (see below). Then the beads were used to infect E. coli. Two pools, therefore, yielded a total of 5 final elutions at the end of the selection.

1st selection set

A. Ligand amplified elution: elute w/ ligand for 1 hr, keep as elution

B. Target amplified elution: elute w/ ligand for 1 hr, toss as wash elute w/ target for 1 hr, keep as elution

C. Bead infect. amp. elution: elute w/ ligand for 1 hr, toss as wash elute w/ target for 1 hr, toss as wash elute w/ cell infection, keep as elution

2nd selection set

A. TEA amplified elution; elute w/ TEA 10min, keep as elution

B. Bead infect. amp. elution; elute w/ TEA 10min, toss as wash elute w/ cell infection, keep as elution

Mid-log prep of XL1 blue MRF' cells for infection
(based on Barbas et al. Phage Display manual procedure)

Culture XL1 blue MRF' in NZCYM (12.5 mg/mL tet) at 37°C and 250 rpm overnight. Started a 500 mL culture in 2 liter flask by diluting cells 1/50 in NZCYM/tet (10 mL overnight culture added) and incubated at 37°C at 250 rpm until OD600 of 0.45 (1.5-2 hrs) was reached. Shaking was reduced to 100 rpm for 10 min. When OD600 reached between 0.55-0.65, cells were transferred to 2 x 250 mL centrifuge bottles, centrifuged at 600 g for 15 min at 4°C. Supernatant

was poured off. Residual liquid was removed with a pipette.

The pellets were gently resuspended (not pipetting up and down) in the original volume of 1 X Minimal A salts at room temp. The resuspended cells were transferred back into 2-liter flask, shaken at 100 rpm for 45 min at 37°C. This process was performed in order to starve the cells and restore pili. The cells were transferred to 2 x 250 mL centrifuge bottles, and centrifuged as earlier.

The cells were gently resuspended in ice cold Minimal A salts (5 mL per 500 mL original culture). The cells were put on ice for use in infections as soon as possible.

The phage eluates were brought up to 7.5 mL with 2XYT medium and 2.5 mL of cells were added. Beads were brought up to 3 mL with 2XYT and 1 mL of cells were added. Incubated at 37oC for 30 min. The cells were plated on 2XYT, 1 mM IPTG agar large NUNC plates and incubated for 18 hr at 30°C.

Example 9: Incorporation of synthetic region in FR1/3 region.

Described below are examples for incorporating of fixed residues in antibody sequences for light chain kappa and lambda genes, and for heavy chains. The experimental conditions and oligonucleotides used for the examples below have been described in previous examples (*e.g.*, Examples 3 & 4).

The process for incorporating fixed FR1 residues in an antibody lambda sequence consists of 3 steps (see FIG. 18): (1) annealing of single-stranded

DNA material encoding VL genes to a partially complementary oligonucleotide mix (indicated with Ext and Bridge), to anneal in this example to the region encoding residues 5-7 of the FR1 of the lambda genes (indicated with X..X; within the lambda genes the overlap may sometimes not be perfect); (2) ligation of this complex; (3) PCR of the ligated material with the indicated primer ('PCRpr') and for example one primer based within the VL gene. In this process the first few residues of all lambda genes will be encoded by the sequences present in the oligonucleotides (Ext., Bridge or PCRpr). After the PCR, the lambda genes can be cloned using the indicated restriction site for ApaLI.

The process for incorporating fixed FR1 residues in an antibody kappa sequence (FIG. 19) consists of 3 steps : (1) annealing of single-stranded DNA material encoding VK genes to a partially complementary oligonucleotide mix (indicated with Ext and Bri), to anneal in this example to the region encoding residues 8-10 of the FR1 of the kappa genes (indicated with X..X; within the kappa genes the overlap may sometimes not be perfect) ; (2) ligation of this complex; (3) PCR of the ligated material with the indicated primer ('PCRpr') and for example one primer based within the VK gene. In this process the first few (8) residues of all kappa genes will be encode by the sequences present in the oligonucleotides (Ext., Bridge or PCRpr.). After the PCR, the kappa genes can be cloned using the indicated restriction site for ApaLI.

The process of incorporating fixed FR3 residues in a antibody heavy chain sequence (FIG. 20) consists of 3 steps : (1) annealing of single-stranded DNA material encoding part of the VH genes (for example encoding FR3, CDR3 and FR4 regions) to a partially

complementary oligonucleotide mix (indicated with Ext and Bridge), to anneal in this example to the region encoding residues 92-94 (within the FR3 region) of VH genes (indicated with X..X; within the VH genes the overlap may sometimes not be perfect); (2) ligation of this complex; (3) PCR of the ligated material with the indicated primer ('PCRpr') and for example one primer based within the VH gene (such as in the FR4 region). In this process certain residues of all VH genes will be encoded by the sequences present in the oligonucleotides used here, in particular from PCRpr (for residues 70-73), or from Ext/Bridge oligonucleotides (residues 74-91). After the PCR, the partial VH genes can be cloned using the indicated restriction site for *XbaI*.

It will be understood that the foregoing is only illustrative of the principles of this invention and that various modifications can be made by those skilled in the art without departing from the scope of and sprit of the invention.

Table 1: Human GLG FR3 sequences

```
! VH1
!  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80
   agg gtc acc atg acc agg gac acg tcc atc agc aca gcc tac atg
!  81  82  82a 82b 82c 83  84  85  86  87  88  89  90  91  92
   gag ctg agc agg ctg aga tct gac gac acg gcc gtg tat tac tgt
!  93  94  95
   gcg aga ga ! 1-02# 1
   aga gtc acc att acc agg gac aca tcc gcg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gaa gac acg gct gtg tat tac tgt
   gcg aga ga ! 1-03# 2
   aga gtc acc atg acc agg aac acc tcc ata agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga gg ! 1-08# 3
   aga gtc acc atg acc aca gac aca tcc acg agc aca gcc tac atg
   gag ctg agg agc ctg aga tct gac gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-18# 4
   aga gtc acc atg acc gag gac aca tct aca gac aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gca aca ga ! 1-24# 5
   aga gtc acc att acc agg gac agg tct atg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac aca gcc atg tat tac tgt
   gca aga ta ! 1-45# 6
   aga gtc acc atg acc agg gac acg tcc acg agc aca gtc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-46# 7
   aga gtc acc att acc agg gac atg tcc aca agc aca gcc tac atg
   gag ctg agc agc ctg aga tcc gag gac acg gcc gtg tat tac tgt
   gcg gca ga ! 1-58# 8
   aga gtc acg att acc gcg gac gaa tcc acg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-69# 9
   aga gtc acg att acc gcg gac aaa tcc acg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-e# 10
   aga gtc acc ata acc gcg gac acg tct aca gac aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gca aca ga ! 1-f# 11
```

! VH2
agg ctc acc atc acc aag gac acc tcc aaa aac cag gtg gtc ctt
aca atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt
gca cac aga c! 2-05# 12
agg ctc acc atc tcc aag gac acc tcc aaa agc cag gtg gtc ctt
acc atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt
gca cgg ata c! 2-26# 13
agg ctc acc atc tcc aag gac acc tcc aaa aac cag gtg gtc ctt
aca atg acc aac atg gac cct gtg gac aca gcc acg tat tac tgt
gca cgg ata c! 2-70# 14
! VH3
cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-07# 15
cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg
caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt
gca aaa gat a! 3-09#16
cga ttc acc atc tcc agg gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 3-11# 17
cga ttc acc atc tcc aga gaa aat gcc aag aac tcc ttg tat ctt
caa atg aac agc ctg aga gcc ggg gac acg gct gtg tat tac tgt
gca aga ga ! 3-13# 18
aga ttc acc atc tca aga gat gat tca aaa aac acg ctg tat ctg
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt
acc aca ga ! 3-15# 19
cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg
caa atg aac agt ctg aga gcc gag gac acg gcc ttg tat cac tgt
gcg aga ga ! 3-20# 20
cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-21# 21
cgg ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gcc gta tat tac tgt
gcg aaa ga ! 3-23# 22
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aaa ga ! 3-30# 23
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt

gcg aga ga ! 3303# 24
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aaa ga ! 3305# 25
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-33# 26
cga ttc acc atc tcc aga gac aac agc aaa aac tcc ctg tat ctg
caa atg aac agt ctg aga act gag gac acc gcc ttg tat tac tgt
gca aaa gat a! 3-43#27
cga ttc acc atc tcc aga gac aat gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gac gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-48# 28
aga ttc acc atc tca aga gat ggt tcc aaa agc atc gcc tat ctg
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt
act aga ga ! 3-49# 29
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 3-53# 30
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg ggc agc ctg aga gct gag gac atg gct gtg tat tac tgt
gcg aga ga ! 3-64# 31
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-66# 32
aga ttc acc atc tca aga gat gat tca aag aac tca ctg tat ctg
caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt
gct aga ga ! 3-72# 33
agg ttc acc atc tcc aga gat gat tca aag aac acg gcg tat ctg
caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt
act aga ca ! 3-73# 34
cga ttc acc atc tcc aga gac aac gcc aag aac acg ctg tat ctg
caa atg aac agt ctg aga gcc gag gac acg gct gtg tat tac tgt
gca aga ga ! 3-74# 35
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg cat ctt
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
aag aaa ga ! 3-d# 36
! VH4
cga gtc acc ata tca gta gac aag tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt

```
gcg aga ga ! 4-04# 37
cga gtc acc atg tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gtg gac acg gcc gtg tat tac tgt
gcg aga aa ! 4-28# 38
cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4301# 39
cga gtc acc ata tca gta gac agg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt
gcc aga ga ! 4302# 40
cga gtt acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gca gac acg gcc gtg tat tac tgt
gcc aga ga ! 4304# 41
cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-31# 42
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gct gtg tat tac tgt
gcg aga ga ! 4-34# 43
cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac tgt
gcg aga ca ! 4-39# 44
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-59# 45
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-61# 46
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gca gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-b# 47
! VH5
cag gtc acc atc tca gcc gac aag tcc atc agc acc gcc tac ctg
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt
gcg aga ca ! 5-51# 48
cac gtc acc atc tca gct gac aag tcc atc agc act gcc tac ctg
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt
gcg aga  ! 5-a# 49
! VH6
cga ata acc atc aac cca gac aca tcc aag aac cag ttc tcc ctg
```

cag ctg aac tct gtg act ccc gag gac acg gct gtg tat tac tgt
gca aga ga ! 6-1# 50

! VH7

cgg ttt gtc ttc tcc ttg gac acc tct gtc agc acg gca tat ctg
cag atc tgc agc cta aag gct gag gac act gcc gtg tat tac tgt
gcg aga ga ! 74.1# 51

Table 2: Enzymes that either cut 15 or more human GLGs or have 5+-base recognition in FR3

Typical entry:

```
REname Recognition                #sites
  GLGid#:base#  GLGid#:base#  GLGid#:base#.....
_____________________________________________
BstEII Ggtnacc                      2
  1:  3   48:  3
 There are   2 hits at base#  3

MaeIII gtnac                       36
  1:  4   2:  4    3:  4    4:  4    5:  4    6:  4
  7:  4   8:  4    9:  4   10:  4   11:  4   37:  4
 37: 58  38:  4   38: 58   39:  4   39: 58   40:  4
 40: 58  41:  4   41: 58   42:  4   42: 58   43:  4
 43: 58  44:  4   44: 58   45:  4   45: 58   46:  4
 46: 58  47:  4   47: 58   48:  4   49:  4   50: 58
 There are  24 hits at base#  4

Tsp45I gtsac                       33
  1:  4   2:  4    3:  4    4:  4    5:  4    6:  4
  7:  4   8:  4    9:  4   10:  4   11:  4   37:  4
 37: 58  38:  4   38: 58   39: 58   40:  4   40: 58
 41: 58  42: 58   43:  4   43: 58   44:  4   44: 58
 45:  4  45: 58   46:  4   46: 58   47:  4   47: 58
 48:  4  49:  4   50: 58
 There are  21 hits at base#  4

HphI tcacc                         45
  1:  5   2:  5    3:  5    4:  5    5:  5    6:  5
  7:  5   8:  5   11:  5   12:  5   12: 11   13:  5
 14:  5  15:  5   16:  5   17:  5   18:  5   19:  5
 20:  5  21:  5   22:  5   23:  5   24:  5   25:  5
 26:  5  27:  5   28:  5   29:  5   30:  5   31:  5
 32:  5  33:  5   34:  5   35:  5   36:  5   37:  5
 38:  5  40:  5   43:  5   44:  5   45:  5   46:  5
 47:  5  48:  5   49:  5
 There are  44 hits at base#  5
```

NlaIII CATG 26

1: 9 1: 42 2: 42 3: 9 3: 42 4: 9
4: 42 5: 9 5: 42 6: 42 6: 78 7: 9
7: 42 8: 21 8: 42 9: 42 10: 42 11: 42
12: 57 13: 48 13: 57 14: 57 31: 72 38: 9
48: 78 49: 78

There are 11 hits at base# 42

There are 1 hits at base# 48 Could cause raggedness.

BsaJI Ccnngg 37

1: 14 2: 14 5: 14 6: 14 7: 14 8: 14
8: 65 9: 14 10: 14 11: 14 12: 14 13: 14
14: 14 15: 65 17: 14 17: 65 18: 65 19: 65
20: 65 21: 65 22: 65 26: 65 29: 65 30: 65
33: 65 34: 65 35: 65 37: 65 38: 65 39: 65
40: 65 42: 65 43: 65 48: 65 49: 65 50: 65
51: 14

There are 23 hits at base# 65

There are 14 hits at base# 14

AluI AGct 42

1: 47 2: 47 3: 47 4: 47 5: 47 6: 47
7: 47 8: 47 9: 47 10: 47 11: 47 16: 63
23: 63 24: 63 25: 63 31: 63 32: 63 36: 63
37: 47 37: 52 38: 47 38: 52 39: 47 39: 52
40: 47 40: 52 41: 47 41: 52 42: 47 42: 52
43: 47 43: 52 44: 47 44: 52 45: 47 45: 52
46: 47 46: 52 47: 47 47: 52 49: 15 50: 47

There are 23 hits at base# 47

There are 11 hits at base# 52 Only 5 bases from 47

BlpI GCtnagc 21

1: 48 2: 48 3: 48 5: 48 6: 48 7: 48
8: 48 9: 48 10: 48 11: 48 37: 48 38: 48
39: 48 40: 48 41: 48 42: 48 43: 48 44: 48
45: 48 46: 48 47: 48

There are 21 hits at base# 48

MwoI GCNNNNNnngc 19

1: 48 2: 28 19: 36 22: 36 23: 36 24: 36
25: 36 26: 36 35: 36 37: 67 39: 67 40: 67
41: 67 42: 67 43: 67 44: 67 45: 67 46: 67
47: 67

There are 10 hits at base# 67
There are 7 hits at base# 36

DdeI Ctnag 71

1: 49 1: 58 2: 49 2: 58 3: 49 3: 58
3: 65 4: 49 4: 58 5: 49 5: 58 5: 65
6: 49 6: 58 6: 65 7: 49 7: 58 7: 65
8: 49 8: 58 9: 49 9: 58 9: 65 10: 49
10: 58 10: 65 11: 49 11: 58 11: 65 15: 58
16: 58 16: 65 17: 58 18: 58 20: 58 21: 58
22: 58 23: 58 23: 65 24: 58 24: 65 25: 58
25: 65 26: 58 27: 58 27: 65 28: 58 30: 58
31: 58 31: 65 32: 58 32: 65 35: 58 36: 58
36: 65 37: 49 38: 49 39: 26 39: 49 40: 49
41: 49 42: 26 42: 49 43: 49 44: 49 45: 49
46: 49 47: 49 48: 12 49: 12 51: 65

There are 29 hits at base# 58
There are 22 hits at base# 49 Only nine base from 58
There are 16 hits at base# 65 Only seven bases from 58

BglII Agatct 11

1: 61 2: 61 3: 61 4: 61 5: 61 6: 61
7: 61 9: 61 10: 61 11: 61 51: 47

There are 10 hits at base# 61

BstYI Rgatcy 12

1: 61 2: 61 3: 61 4: 61 5: 61 6: 61
7: 61 8: 61 9: 61 10: 61 11: 61 51: 47

There are 11 hits at base# 61

Hpy188I TCNga 17

1: 64 2: 64 3: 64 4: 64 5: 64 6: 64

7: 64 8: 64 9: 64 10: 64 11: 64 16: 57

20: 57 27: 57 35: 57 48: 67 49: 67

There are 11 hits at base# 64

There are 4 hits at base# 57

There are 2 hits at base# 67 Could be ragged.

MslI CAYNNnnRTG 44

1: 72 2: 72 3: 72 4: 72 5: 72 6: 72

7: 72 8: 72 9: 72 10: 72 11: 72 15: 72

17: 72 18: 72 19: 72 21: 72 23: 72 24: 72

25: 72 26: 72 28: 72 29: 72 30: 72 31: 72

32: 72 33: 72 34: 72 35: 72 36: 72 37: 72

38: 72 39: 72 40: 72 41: 72 42: 72 43: 72

44: 72 45: 72 46: 72 47: 72 48: 72 49: 72

50: 72 51: 72

There are 44 hits at base# 72

BsiEI CGRYcg 23

1: 74 3: 74 4: 74 5: 74 7: 74 8: 74

9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

33: 74 34: 74 37: 74 38: 74 39: 74 40: 74

41: 74 42: 74 45: 74 46: 74 47: 74

There are 23 hits at base# 74

EaeI Yggccr 23

1: 74 3: 74 4: 74 5: 74 7: 74 8: 74

9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

33: 74 34: 74 37: 74 38: 74 39: 74 40: 74

41: 74 42: 74 45: 74 46: 74 47: 74

There are 23 hits at base# 74

EagI Cggccg 23

1: 74 3: 74 4: 74 5: 74 7: 74 8: 74

9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

33: 74 34: 74 37: 74 38: 74 39: 74 40: 74

41: 74	**42: 74**	**45: 74**	**46: 74**	**47: 74**

There are 23 hits at base# 74

HaeIII GGcc 27

1: 75	3: 75	4: 75	5: 75	7: 75	8: 75
9: 75	10: 75	11: 75	16: 75	17: 75	20: 75
22: 75	30: 75	33: 75	34: 75	37: 75	38: 75
39: 75	40: 75	41: 75	42: 75	45: 75	46: 75
47: 75	48: 63	49: 63			

There are 25 hits at base# 75

Bst4CI ACNgt 65°C 63 Sites There is a third isoschismer

1: 86	**2: 86**	**3: 86**	**4: 86**	**5: 86**	**6: 86**
7: 34	**7: 86**	**8: 86**	**9: 86**	**10: 86**	**11: 86**
12: 86	**13: 86**	**14: 86**	**15: 36**	**15: 86**	**16: 53**
16: 86	**17: 36**	**17: 86**	**18: 86**	**19: 86**	**20: 53**
20: 86	**21: 36**	**21: 86**	**22: 0**	**22: 86**	**23: 86**
24: 86	**25: 86**	**26: 86**	**27: 53**	**27: 86**	**28: 36**
28: 86	**29: 86**	**30: 86**	**31: 86**	**32: 86**	**33: 36**
33: 86	**34: 86**	**35: 53**	**35: 86**	**36: 86**	**37: 86**
38: 86	**39: 86**	**40: 86**	**41: 86**	**42: 86**	**43: 86**
44: 86	**45: 86**	**46: 86**	**47: 86**	**48: 86**	**49: 86**
50: 86	**51: 0**	**51: 86**			

There are 51 hits at base# 86 All the other sites are well away

HpyCH4III ACNgt 63

1: 86	**2: 86**	**3: 86**	**4: 86**	**5: 86**	**6: 86**
7: 34	**7: 86**	**8: 86**	**9: 86**	**10: 86**	**11: 86**
12: 86	**13: 86**	**14: 86**	**15: 36**	**15: 86**	**16: 53**
16: 86	**17: 36**	**17: 86**	**18: 86**	**19: 86**	**20: 53**
20: 86	**21: 36**	**21: 86**	**22: 0**	**22: 86**	**23: 86**
24: 86	**25: 86**	**26: 86**	**27: 53**	**27: 86**	**28: 36**
28: 86	**29: 86**	**30: 86**	**31: 86**	**32: 86**	**33: 36**
33: 86	**34: 86**	**35: 53**	**35: 86**	**36: 86**	**37: 86**
38: 86	**39: 86**	**40: 86**	**41: 86**	**42: 86**	**43: 86**
44: 86	**45: 86**	**46: 86**	**47: 86**	**48: 86**	**49: 86**
50: 86	**51: 0**	**51: 86**			

There are 51 hits at base# 86

HinfI Gantc **43**

2: 2 3: 2 4: 2 5: 2 6: 2 7: 2
8: 2 9: 2 9: 22 10: 2 11: 2 15: 2
16: 2 17: 2 18: 2 19: 2 19: 22 20: 2
21: 2 23: 2 24: 2 25: 2 26: 2 27: 2
28: 2 29: 2 30: 2 31: 2 32: 2 33: 2
33: 22 34: 22 35: 2 36: 2 37: 2 38: 2
40: 2 43: 2 44: 2 45: 2 46: 2 47: 2
50: 60

There are 38 hits at base# 2

MlyI GAGTCNNNNNn 18

2: 2 3: 2 4: 2 5: 2 6: 2 7: 2
8: 2 9: 2 10: 2 11: 2 37: 2 38: 2
40: 2 43: 2 44: 2 45: 2 46: 2 47: 2
There are 18 hits at base# 2

PleI gagtc **18**

2: 2 3: 2 4: 2 5: 2 6: 2 7: 2
8: 2 9: 2 10: 2 11: 2 37: 2 38: 2
40: 2 43: 2 44: 2 45: 2 46: 2 47: 2
There are 18 hits at base# 2

AciI Ccgc 24

2: 26 9: 14 10: 14 11: 14 27: 74 37: 62
37: 65 38: 62 39: 65 40: 62 40: 65 41: 65
42: 65 43: 62 43: 65 44: 62 44: 65 45: 62
46: 62 47: 62 47: 65 48: 35 48: 74 49: 74

There are 8 hits at base# 62
There are 8 hits at base# 65
There are 3 hits at base# 14
There are 3 hits at base# 74
There are 1 hits at base# 26
There are 1 hits at base# 35

-"- Gcgg 11

8: 91 9: 16 10: 16 11: 16 37: 67 39: 67

```
 40: 67   42: 67   43: 67   45: 67   46: 67
 There are   7 hits at base# 67
 There are   3 hits at base# 16
 There are   1 hits at base# 91

BsiHKAI GWGCWc                  20
  2: 30    4: 30    6: 30    7: 30    9: 30   10: 30
 12: 89   13: 89   14: 89   37: 51   38: 51   39: 51
 40: 51   41: 51   42: 51   43: 51   44: 51   45: 51
 46: 51   47: 51
 There are  11 hits at base# 51

Bsp1286I GDGCHc                 20
  2: 30    4: 30    6: 30    7: 30    9: 30   10: 30
 12: 89   13: 89   14: 89   37: 51   38: 51   39: 51
 40: 51   41: 51   42: 51   43: 51   44: 51   45: 51
 46: 51   47: 51
 There are  11 hits at base# 51

HgiAI GWGCWc                    20
  2: 30    4: 30    6: 30    7: 30    9: 30   10: 30
 12: 89   13: 89   14: 89   37: 51   38: 51   39: 51
 40: 51   41: 51   42: 51   43: 51   44: 51   45: 51
 46: 51   47: 51
 There are  11 hits at base# 51

BsoFI GCngc                     26
  2: 53    3: 53    5: 53    6: 53    7: 53    8: 53
  8: 91    9: 53   10: 53   11: 53   31: 53   36: 36
 37: 64   39: 64   40: 64   41: 64   42: 64   43: 64
 44: 64   45: 64   46: 64   47: 64   48: 53   49: 53
 50: 45   51: 53
 There are  13 hits at base# 53
 There are  10 hits at base# 64

TseI Gcwgc                      17
  2: 53    3: 53    5: 53    6: 53    7: 53    8: 53
  9: 53   10: 53   11: 53   31: 53   36: 36   45: 64
```

46: 64 48: 53 49: 53 50: 45 51: 53
There are 13 hits at base# 53

MnlI gagg 34

3: 67 3: 95 4: 51 5: 16 5: 67 6: 67
7: 67 8: 67 9: 67 10: 67 11: 67 15: 67
16: 67 17: 67 19: 67 20: 67 21: 67 22: 67
23: 67 24: 67 25: 67 26: 67 27: 67 28: 67
29: 67 30: 67 31: 67 32: 67 33: 67 34: 67
35: 67 36: 67 50: 67 51: 67
There are 31 hits at base# 67

HpyCH4V TGca 34

5: 90 6: 90 11: 90 12: 90 13: 90 14: 90
15: 44 16: 44 16: 90 17: 44 18: 90 19: 44
20: 44 21: 44 22: 44 23: 44 24: 44 25: 44
26: 44 27: 44 27: 90 28: 44 29: 44 33: 44
34: 44 35: 44 35: 90 36: 38 48: 44 49: 44
50: 44 50: 90 51: 44 51: 52
There are 21 hits at base# 44
There are 1 hits at base# 52

AccI GTmkac 13 5-base recognition
7: 37 11: 24 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 43: 16 44: 16 45: 16 46: 16
47: 16
There are 11 hits at base# 16

SacII CCGCgg 8 6-base recognition
9: 14 10: 14 11: 14 37: 65 39: 65 40: 65
42: 65 43: 65
There are 5 hits at base# 65
There are 3 hits at base# 14

TfiI Gawtc 24
9: 22 15: 2 16: 2 17: 2 18: 2 19: 2
19: 22 20: 2 21: 2 23: 2 24: 2 25: 2

26: 2 27: 2 28: 2 29: 2 30: 2 31: 2
32: 2 33: 2 33: 22 34: 22 35: 2 36: 2
There are 20 hits at base# 2

BsmAI Nnnnnngagac 19
15: 11 16: 11 20: 11 21: 11 22: 11 23: 11
24: 11 25: 11 26: 11 27: 11 28: 11 28: 56
30: 11 31: 11 32: 11 35: 11 36: 11 44: 87
48: 87
There are 16 hits at base# 11

BpmI ctccag 19
15: 12 16: 12 17: 12 18: 12 20: 12 21: 12
22: 12 23: 12 24: 12 25: 12 26: 12 27: 12
28: 12 30: 12 31: 12 32: 12 34: 12 35: 12
36: 12
There are 19 hits at base# 12

XmnI GAANNnnttc 12
37: 30 38: 30 39: 30 40: 30 41: 30 42: 30
43: 30 44: 30 45: 30 46: 30 47: 30 50: 30
There are 12 hits at base# 30

BsrI NCcagt 12
37: 32 38: 32 39: 32 40: 32 41: 32 42: 32
43: 32 44: 32 45: 32 46: 32 47: 32 50: 32
There are 12 hits at base# 32

BanII GRGCYc 11
37: 51 38: 51 39: 51 40: 51 41: 51 42: 51
43: 51 44: 51 45: 51 46: 51 47: 51
There are 11 hits at base# 51

Ecl136I GAGctc 11
37: 51 38: 51 39: 51 40: 51 41: 51 42: 51
43: 51 44: 51 45: 51 46: 51 47: 51
There are 11 hits at base# 51

```
SacI GAGCTc                    11
 37: 51   38: 51   39: 51   40: 51   41: 51   42: 51
 43: 51   44: 51   45: 51   46: 51   47: 51
 There are  11 hits at base# 51
```

Table 3: Synthetic 3-23 FR3 of human heavy chains showning positions of possible cleavage sites

```
! Sites engineered into the synthetic gene are shown in upper case
DNA
! with the RE name between vertical bars (as in | XbaI  |).
! RERSs frequently found in GLGs are shown below the synthetic
sequence
! with the name to the right (as in gtn ac=MaeIII(24), indicating
that
! 24 of the 51 GLGs contain the site).
!
!                                                          |---FR3---
!                                                           89  90 (codon
# in
!                                                            R   F
synthetic 3-23)
                                                          |cgc|ttc|  6
!  Allowed DNA                                            |cgn|tty|
!                                                         |agr|
!                                                           ga ntc =
HinfI(38)
!                                                           ga gtc =
PleI(18)
!                                                           ga wtc =
TfiI(20)
!                                                              gtn ac =
MaeIII(24)
!                                                              gts ac =
Tsp45I(21)
!                                                               tc acc =
HphI(44)
!
!       --------FR3-----------------------------------------------------
!         91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!          T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
          |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
51
!allowed|acn|ath|tcn|cgn|gay|aay|tcn|aar|aay|acn|ttr|tay|ttr|car|atg|
!               |agy|agr|       |agy|           |ctn|   |ctn|
!               |     ga|gac = BsmAI(16)                    ag ct =
AluI(23)
```

```
!               c|tcc ag = BpmI(19)                              g ctn agc =
BlpI(21)
!                |       |               g aan nnn ttc = XmnI(12)
!                | XbaI  |                                   tg ca =
HpyCH4V(21)
!
!       ---FR3--------------------------------------------------->|
!        106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!         N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   K
        |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|  96
!allowed|aay|tcn|ttr|cgn|gcn|gar|gay|acn|gcn|gtn|tay|tay|tgy|gcn|aar|
!           |agy|ctn|agr|               |       |
!               |       |  cc nng g = BsaJI(23)      ac ngt = Bst4CI(51)
!               |     aga tct = BglII(10)   |        ac ngt =
HpyCH4III(51)
!               |     Rga tcY = BstYI(11)   |        ac ngt = TaaI(51)
!               |       |           c ayn nnn rtc = MslI(44)
!               |       |              cg ryc g = BsiEI(23)
!               |       |              yg gcc r = EaeI(23)
!               |       |              cg gcc g = EagI(23)
!               |       |              |g gcc = HaeIII(25)
!               |       |     gag g = MnlI(31)|
!               |AflII  |               | PstI |
```

Table 4: REdaptors, Extenders, and Bridges used for Cleavage and Capture of Human Heavy Chains in FR3.

A: HpyCH4V Probes of actual human HC genes

```
 !HpyCH4V in FR3 of human HC, bases 35-56; only those with TGca site
 TGca;10,
 RE recognition:tgca                          of length  4 is expected at
10
   1                                      6-1  agttctccctgcagctgaactc
   2                 3-11,3-07,3-21,3-72,3-48  cactgtatctgcaaatgaacag
   3                           3-09,3-43,3-20  ccctgtatctgcaaatgaacag
   4                                    5-51  ccgcctacctgcagtggagcag
   5  3-15,3-30,3-30.5,3-30.3,3-74,3-23,3-33  cgctgtatctgcaaatgaacag
   6                                    7-4.1  cggcatatctgcagatctgcag
   7                                     3-73  cggcgtatctgcaaatgaacag
   8                                      5-a  ctgcctacctgcagtggagcag
   9                                     3-49  tcgcctatctgcaaatgaacag
```

B: HpyCH4V REdaptors, Extenders, and Bridges

B.1 REdaptors

! Cutting HC lower strand:			
! TmKeller for 100 mM NaCl, zero formamide			
! Edapters for cleavage		T_m^W	T_m^K
(ON_HCFR36-1)	5'-agttctcccTGCAgctgaactc-3'	68.0	64.5
(ON_HCFR36-1A)	5'-ttctcccTGCAgctgaactc-3'	62.0	62.5
(ON_HCFR36-1B)	5'-ttctcccTGCAgctgaac-3'	56.0	59.9
(ON_HCFR33-15)	5'-cgctgtatcTGCAaatgaacag-3'	64.0	60.8
(ON_HCFR33-15A)	5'-ctgtatcTGCAaatgaacag-3'	56.0	56.3
(ON_HCFR33-15B)	5'-ctgtatcTGCAaatgaac-3'	50.0	53.1
(ON_HCFR33-11)	5'-cactgtatcTGCAaatgaacag-3'	62.0	58.9
(ON_HCFR35-51)	5'-ccgcctaccTGCAgtggagcag-3'	74.0	70.1
!			

B.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

```
!                    XbaI...
!D323*  cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC
!       scab..... designed gene 3-23 gene.............
!
!    HpyCH4V
!     .. ..            AflII...
```

! **Ttg caG** atg aac agc TtA agG . . .
! ....
!

B.3 Extender and Bridges

! Extender (bottom strand):
!
(ON_HCHpyEx01) 5'-cAAgTAgAgAgTATTcTTAgAgTTgTcTcTAgAcTTAgTgAAgcg-3'
! **ON_HCHpyEx01 is the reverse complement of**
! 5'-cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC **Ttg** -3'
!
! Bridges (top strand, 9-base overlap):
!
(ON_HCHpyBr016-1) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
aaT acT ctC taC **Ttg** CAgctgaac-3' {3'-term C is
blocked}
!
! 3-15 et al. + 3-11
(ON_HCHpyBr023-15) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
aaT acT ctC taC **Ttg** CAaatgaac-3' {3'-term C is
blocked}
!

! 5-51
(ON_HCHpyBr045-51) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
aaT acT ctC taC **Ttg** CAgtggagc-3' {3'-term C is
blocked}
!
! PCR primer (top strand)
!
(ON_HCHpyPCR) 5'-cgCttcacTaag tcT aga gac-3'
!

C: *BlpI* Probes from human HC GLGs

1 1-58,1-03,1-08,1-69,1-24,1-45,1-46,1-f,1-e
acatggaGCTGAGCagcctgag
2 1-02
acatggaGCTGAGCaggctgag
3 1-18
acatggagctgaggagcctgag

4 5-51,5-a
acctgcagtggagcagcctgaa
5 3-15,3-73,3-49,3-72
atctgcaaatgaacagcctgaa
6 3303,3-33,3-07,3-11,3-30,3-21,3-23,3305,3-48
atctgcaaatgaacagcctgag
7 3-20,3-74,3-09,3-43
atctgcaaatgaacagtctgag
8 74.1
atctgcagatctgcagcctaaa
9 3-66,3-13,3-53,3-d
atcttcaaatgaacagcctgag
10 3-64
atcttcaaatgggcagcctgag
11 4301,4-28,4302,4-04,4304,4-31,4-34,4-39,4-59,4-61,4-b
ccctgaaGCTGAGCtctgtgac
12 6-1
ccctgcagctgaactctgtgac
13 2-70,2-05
tccttacaatgaccaacatgga
14 2-26
tccttaccatgaccaacatgga

D: *BlpI* REdaptors, Extenders, and Bridges

D.1 REdaptors

		T_m^W	T_mK
(BlpF3HC1-58)	5'-ac atg ga**G CTG AGC** agc ctg ag-3'	70	66.4
(BlpF3HC6-1)	5'-cc ctg aag ctg agc tct gtg ac-3'	70	66.4

! BlpF3HC6-1 matches 4-30.1, not 6-1.

D.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

!
BlpI
! XbaI...
... ...
!D323* cgCttcacTaag TCT AGA gac aaC tcT aag aaT acT ctC taC Ttg caG atg aac

```
!
!                    AflII...
!                  agC TTA AGG
```

D.3 Extender and Bridges

```
! Bridges
(BlpF3Br1) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                  taC Ttg caG Ctg a|GC agc ctg-3'
(BlpF3Br2) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                  taC Ttg caG Ctg a|gc tct gtg-3'
!                                  | lower strand is cut here
! Extender
(BlpF3Ext) 5'-
TcAgcTgcAAgTAcAAAgTATTTTTAcTgTTATcTcTAgAcTgAgTgAAgcg-3'
! BlpF3Ext is the reverse complement of:
! 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG taC Ttg caG
Ctg a-3'
!
(BlpF3PCR) 5'-cgCttcacTcag tcT aga gaT aaC-3'
```

E: *HpyCH4III* Distinct GLG sequences surrounding site, bases 77-98

```
   1          102#1,118#4,146#7,169#9,1e#10,311#17,353#30,404#37,4301
ccgtgtattactgtgcgagaga
   2          103#2,307#15,321#21,3303#24,333#26,348#28,364#31,366#32
ctgtgtattactgtgcgagaga
   3                                                          108#3
ccgtgtattactgtgcgagagg
   4                                                  124#5,1f#11
ccgtgtattactgtgcaacaga
   5                                                          145#6
ccatgtattactgtgcaagata
   6                                                          158#8
ccgtgtattactgtgcggcaga
   7                                                         205#12
ccacatattactgtgcacacag
   8                                                         226#13
ccacatattactgtgcacggat
   9                                                         270#14
ccacgtattactgtgcacggat
```

10 309#16,343#27
ccttgtattactgtgcaaaaga
11 313#18,374#35,61#50
ctgtgtattactgtgcaagaga
12 315#19
ccgtgtattactgtaccacaga
13 320#20
ccttgtatcactgtgcgagaga
14 323#22
ccgtatattactgtgcgaaaga
15 330#23,3305#25
ctgtgtattactgtgcgaaaga
16 349#29
ccgtgtattactgtactagaga
17 372#33
ccgtgtattactgtgctagaga
18 373#34
ccgtgtattactgtactagaca
19 3d#36
ctgtgtattactgtaagaaaga
20 428#38
ccgtgtattactgtgcgagaaa
21 4302#40,4304#41
ccgtgtattactgtgccagaga
22 439#44
ctgtgtattactgtgcgagaca
23 551#48
ccatgtattactgtgcgagaca
24 5a#49
ccatgtattactgtgcgaga

F: *Hpy*CH4III REdaptors, Extenders, and Bridges

F.1 REdaptors

! ONs for cleavage of HC(lower) in FR3(bases 77-97)
! For cleavage with HpyCH4III, Bst4CI, or TaaI
! cleavage is in lower chain before base 88.

!	77 788 888 888 889 999 999 9		
!	78 901 234 567 890 123 456 7	T_m^W	T_m^K
(H43.77.97.1-02#1)	5'-cc gtg tat tAC TGT gcg aga g-3'	6462.6	
(H43.77.97.1-03#2)	5'-c[t] gtg tat tAC TGT gcg aga g-3'	6260.6	
(H43.77.97.108#3)	5'-cc gtg tat tAC TGT gcg aga g-3'	6462.6	
(H43.77.97.323#22)	5'-cc gt[a] tat tac tgt gcg a[a]a g-3'	6058.7	
(H43.77.97.330#23)	5'-c[t] gtg tat tac tgt gcg a[a]a g-3'	6058.7	
(H43.77.97.439#44)	5'-c[t] gtg tat tac tgt gcg aga [c]-3'	6260.6	

(H43.77.97.551#48) 5'-cc atg tat tac tgt gcg aga c-3' 6260.6
(H43.77.97.5a#49) 5'-cc atg tat tAC TGT gcg aga c-3' 5858.3

F.2 Extender and Bridges

! XbaI and AflII sites in bridges are bunged
(H43.XABr1) 5'-ggtgtagtga-
|TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3'
(H43.XABr2) 5'-ggtgtagtga-
|TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3'
(H43.XAExt) 5'-ATAgTAgAcT gcAgTgTccT cAgcccTTAA gcTgTTcATc
TgcAAgTAgA-
gAgTATTcTT AgAgTTgTcT cTAgATcAcT AcAcc-3'
!H43.XAExt is the reverse complement of
! 5'-ggtgtagtga-
! **|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-**
! **|aac|agC|TTA|AGg|gct|gag|gac|**aCT|GCA|Gtc|tac|tat -3'

(H43.XAPCR) 5'-ggtgtagtga **|TCT|AGA|gac|aac-3'**
! XbaI and AflII sites in bridges are bunged
(H43.ABr1) 5'-ggtgtagtga-
|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3'
(H43.ABr2) 5'-ggtgtagtga-
|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3'
(H43.AExt) 5'-ATAgTAgAcTgcAgTgTccTcAgcccTTAAgcTgTTTcAcTAcAcc-3'
!(H43.AExt) is the reverse complement of 5'-ggtgtagtga-
! **|aac|agC|TTA|AGg|gct|gag|gac|**aCT|GCA|Gtc|tac|tat -3'
(H43.APCR) 5'-ggtgtagtga **|aac|agC|TTA|AGg|gct|g-3'**

Table 5: Analysis of frequency of matching REdaptors in actual V genes

A: HpyCH4V in HC at bases 35-56

			Number of mismatches.....										Number		
	Id	Ntot	0	1	2	3	4	5	6	7	8	9	10	Cut	Id	Probe
	1	**510**	**5**	**11**	**274**	**92**	**61**	**25**	**22**	**11**	**1**	**3**	**5**	**443**	**6-1**	**agttctcccTGCAgctgaactc**
	2	**192**	**54**	**42**	**32**	**24**	**15**	**2**	**3**	**10**	**3**	**1**	**6**	**167**	**3-11**	**cactgtatcTGCAaatgaacag**
	3	58	19	7	17	6	5	1	0	1	0	2	0	54	3-09	ccctgtatcTGCAaatgaacag
	4	**267**	**42**	**33**	**9**	**8**	**8**	**82**	**43**	**22**	**8**	**11**	**1**	**100**	**5-51**	**ccgcctaccTGCAgtggagcag**
	5	**250**	**111**	**59**	**41**	**24**	**7**	**5**	**1**	**0**	**0**	**2**	**0**	**242**	**3-15**	**cgctgtatcTGCAaatgaacag**
	6	7	0	2	0	1	0	0	0	0	0	4	0	3	7-4.1	cggcatatcTGCAgatctgcag
	7	7	0	2	2	0	0	2	1	0	0	0	0	4	3-73	cggcgtatcTGCAaatgaacag
	8	26	10	4	1	3	1	2	1	3	1	0	0	19	5-a	ctgcctaccTGCAgtggagcag
	9	21	8	2	3	1	6	1	0	0	0	0	0	20	3-49	tcgcctatcTGCAaatgaacag
		1338	249	162	379	149	103	120	71	47	13	23	12	1052		
			249	411	790	939		1162		1280		1316				
							1042		1233		1293		1338			

Id	Probe	dotted probe
6-1	agttctcccTGCAgctgaactc	agttctccc**TGCA**gctgaactc
3-11	cactgtatcTGCAaatgaacag	cac.g.at.....aa.....ag
3-09	ccctgtatcTGCAaatgaacag	ccc.g.at.....aa.....ag
5-51	ccgcctaccTGCAgtggagcag	ccgc..a.......tg..g.ag

3-15	cgctgtatcTGCAaatgaacag	c.c.g.at.....aa.....ag
7-4.1	cggcatatcTGCAgatctgcag	c.gca.at......a.ctg.ag
3-73	cggcgtatcTGCAaatgaacag	c.gcg.at.....aa.....ag
5-a	ctgcctaccTGCAgtggagcag	ctgc..a.......tg..g.ag
3-49	tcgcctatcTGCAaatgaacag	tcgc..at.....aa.....ag

Seqs with the expected RE site only.....1004
(Counts only cases with 4 or fewer mismatches)
Seqs with only an unexpected site..... 0
Seqs with both expected and unexpected.... 48
(Counts only cases with 4 or fewer mismatches)
Seqs with no sites..... 0

B: BlpI in HC

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	Name	
1	**133**	**73**	**16**	**11**	**13**	**6**	**9**	**1**	**4**	**0**	**119**	**1-58**	**acatggaGCTGAGCagcctgag**
2	14	11	1	0	0	0	0	1	0	1	12	1-02	acatgga**gctgagc**aggctgag
3	34	17	8	2	6	1	0	0	0	0	0	1-18	acatggagctgaggagcctgag
4	120	50	32	16	10	9	1	1	1	0	2	5-51	acctgcagtggagcagcctgaa
5	55	13	11	10	17	3	1	0	0	0	0	3-15	atctgcaaatgaacagcctgaa
6	340	186	88	41	15	6	3	0	1	0	0	3303	atctgcaaatgaacagcctgag
7	82	25	16	25	12	1	3	0	0	0	0	3-20	atctgcaaatgaacagtctgag

	8	3	0	2	0	1	0	0	0	0	0	0	74.1	atctgcagatctgcagcctaaa
	9	23	18	2	2	1	0	0	0	0	0	0	3-66	atcttcaaatgaacagcctgag
	10	2	1	0	1	0	0	0	0	0	0	0	3-64	atcttcaaatgggcagcctgag
	11	**486**	**249**	**78**	**81**	**38**	**21**	**10**	**4**	**4**	**1**	**467**	**4301**	**ccctgaagctgagctctgtgac**
5	12	16	6	3	1	0	1	1	3	1	0	1	6-1	ccctgcagctgaactctgtgac
	13	28	15	8	2	2	1	0	0	0	0	0	2-70	tccttacaatgaccaacatgga
	14	2	0	2	0	0	0	0	0	0	0	0	2-26	tccttaccatgaccaacatgga
												601		

	Name	Full sequence	Dot mode
	1-58	acatggaGCTGAGCagcctgag	acatggaGCTGAGCagcctgag
	1-02	acatgga**gctgagc**aggctgag	..............g.....
	1-18	acatggagctgaggagcctgag	............g........
5	5-51	acctgcagtggagcagcctgaa	..c..c..tg..........a
	3-15	atctgcaaatgaacagcctgaa	.tc..c.aa...a........a
	3-30.3	atctgcaaatgaacagcctgag	.tc..c.aa...a.........
	3-20	atctgcaaatgaacagtctgag	.tc..c.aa...a...t.....
	7-4.1	atctgcagatctgcagcctaaa	.tc..c..a.ct.......a.a
10	3-66	atcttcaaatgaacagcctgag	.tc.tc.aa...a.........
	3-64	atcttcaaatgggcagcctgag	.tc.tc.aa..g..........
	4-30.1	ccctgaagctgagctctgtgac	c.c..a........tctg...c
	6-1	ccctgcagctgaactctgtgac	c.c..c......a.tctg...c
	2-70	tccttacaatgaccaacatgga	t.c.tacaa...c..a.a..ga
15	2-26	tccttaccatgaccaacatgga	t.c.tacca...c..a.a..ga

Seqs with the expected RE site only..... 597 (counting sequences with 4 or fewer mismatches)

Seqs with only an unexpected site..... 2

Seqs with both expected and unexpected.... 2

Seqs with no sites..... 686

20 C: HpyCH4III, Bst4CI, or TaaI in HC

In scoring whether the RE site of interest is present, only ONs that have 4 or fewer mismatches are counted.

Number of sequences.......... 1617

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut		acngt	acngt
1	**244**	**78**	**92**	**43**	**18**	**10**	**1**	**2**	**0**	**0**	**241**	**102#1,1**	**ccgtgtattACTGTgcgagaga**	ccgtgtattactgtgcgagaga
2	**457**	**69**	**150**	**115**	**66**	**34**	**11**	**8**	**3**	**1**	**434**	**103#2,3**	**ctgtgtattactgtgcgagaga**	.t..................
3	**173**	**52**	**45**	**36**	**22**	**14**	**3**	**0**	**0**	**1**	**169**	**108#3**	**ccgtgtattactgtgcgagagg**	.....................g
4	16	0	3	2	2	1	6	0	1	1	8	124#5,1	ccgtgtattactgtgcaacaga	..............a.c...
5	4	0	0	1	0	1	1	0	1	0	2	145#6	ccatgtattactgtgcaagata	..a.............a...t.
6	15	1	0	1	0	6	4	1	1	1	8	158#8	ccgtgtattactgtgcggcaga	................gc...
7	23	4	8	5	2	2	1	1	0	0	21	205#12	ccacatattactgtgcacacag	..aca..........acacag
8	9	1	1	1	0	3	2	1	0	0	6	226#13	ccacatattactgtgcacggat	..aca..........ac.gat
9	7	1	3	1	1	0	0	1	0	0	6	270#14	ccacgtattactgtgcacggat	..ac............ac.gat
10	23	7	3	5	5	2	1	0	0	0	22	309#16,	ccttgtattactgtgcaaaaga	..t.............a.a...
11	35	5	10	7	6	3	3	0	1	0	31	313#18,	ctgtgtattactgtgcaagaga	.t..............a.....
12	18	2	3	2	2	6	1	0	2	0	15	315#19	ccgtgtattactgtaccacaga	.............a.c.c...
13	3	1	2	0	0	0	0	0	0	0	3	320#20	ccttgtatcactgtgcgagaga	..t.....c.............
14	**117**	**29**	**23**	**28**	**22**	**8**	**4**	**2**	**1**	**0**	**110**	**323#22**	**ccgtatattactgtgcgaaaga**	....a.............a...
15	**75**	**21**	**25**	**13**	**9**	**1**	**4**	**2**	**0**	**0**	**69**	**330#23,**	**ctgtgtattactgtgcgaaaga**	.t................a...
16	14	2	2	2	3	0	3	1	1	0	9	349#29	ccgtgtattactgtactagaga	.............a.t.....
17	2	0	0	1	0	0	1	0	0	0	1	372#33	ccgtgtattactgtgctagaga	................t.....
18	1	0	0	1	0	0	0	0	0	0	1	373#34	ccgtgtattactgtactagaca	.............a.t...c.
19	2	0	0	0	0	0	0	0	0	2	0	3d#36	ctgtgtattactgtaagaaaga	.t............aa..a...
20	34	4	9	9	4	5	3	0	0	0	31	428#38	ccgtgtattactgtgcgagaaa	...................a.
21	17	5	4	2	2	3	1	0	0	0	16	4302#40	ccgtgtattactgtgccagaga	................c.....
22	**75**	**15**	**17**	**24**	**7**	**10**	**1**	**1**	**0**	**0**	**73**	**439#44**	**ctgtgtattactgtgcgagaca**	.t.................c.
23	**40**	**14**	**15**	**4**	**5**	**1**	**0**	**1**	**0**	**0**	**39**	**551#48**	**ccatgtattactgtgcgagaca**	..a................c.

24	213	26	56	60	42	20	7	2	0	0	204	5a#49
ccatgtattactgtgcgagaAA		..a.....AA										
Group		337	471	363	218	130	58	23	11	6		
Cumulative		337	808	1171	1389	1519	1577	1600	1611	1617		

Seqs with the expected RE site only.....1511
Seqs with only an unexpected site..... 0
Seqs with both expected and unexpected.... 8
Seqs with no sites..... 0

Table 5D:

Analysis repeated using only 8 best REdaptors

Id	Ntot	0	1	2	3	4	5	6	7	8+	
1	301	78	101	54	32	16	9	10	1	0	281 102#1

ccgtgtattactgtgcgagaga

2	493	69	155	125	73	37	14	11	3	6	459 103#2

ctgtgtattactgtgcgagaga

3	189	52	45	38	23	18	5	4	1	3	176 108#3

ccgtgtattactgtgcgagagg

4	127	29	23	28	24	10	6	5	2	0	114 323#22

ccgtatattactgtgcgaaaga

5	78	21	25	14	11	1	4	2	0	0	72 330#23

ctgtgtattactgtgcgaaaga 6 79 15 17 25 8 11 1 2 0 0 76 439#44 ctgtgtattactgtgcgagaca

7	43	14	15	5	5	3	0	1	0	0	42 551#48

ccatgtattactgtgcgagaca

8	307	26	63	72	51	38	24	14	13	6	250 5a#49

ccatgtattactgtgcgaga

1	102#1	ccgtgtattactgtgcgagaga	ccgtgtattactgtgcgagaga
2	103#2	ctgtgtattactgtgcgagaga	.t..................
3	108#3	ccgtgtattactgtgcgagagg	..................g
4	323#22	ccgtatattactgtgcgaaaga	....a.............a...
5	330#23	ctgtgtattactgtgcgaaaga	.t..............a...
6	439#44	ctgtgtattactgtgcgagaca	.t................c.
7	551#48	ccatgtattactgtgcgagaca	..a..............c.
8	5a#49	ccatgtattactgtgcgagaAA	..a..............AA

Seqs with the expected RE site only.....1463 / 1617
Seqs with only an unexpected site..... 0
Seqs with both expected and unexpected.... 7
Seqs with no sites..... 0

Table 6: Human HC GLG FR1 Sequences

VH Exon - Nucleotide sequence alignment

VH1

1-02 CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG
GTC TCC TGC AAG GCT TCT GGA TAC ACC TTC ACC

1-03 cag gtC cag ctT gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtT tcc tgc aag gct tct gga tac acc ttc acT

1-08 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtc tcc tgc aag gct tct gga tac acc ttc acc

1-18 cag gtT cag ctg gtg cag tct ggA gct gag gtg aag aag cct ggg gcc tca gtg aag
gtc tcc tgc aag gct tct ggT tac acc ttT acc

1-24 cag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtc tcc tgc aag gTt tcC gga tac acc Ctc acT

1-45 cag Atg cag ctg gtg cag tct ggg gct gag gtg aag aag Act ggg Tcc tca gtg aag
gtT tcc tgc aag gct tcC gga tac acc ttc acc

1-46 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtT tcc tgc aag gcA tct gga tac acc ttc acc

1-58 caA Atg cag ctg gtg cag tct ggg Cct gag gtg aag aag cct ggg Acc tca gtg aag
gtc tcc tgc aag gct tct gga tTc acc ttT acT

1-69 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag
gtc tcc tgc aag gct tct gga GGc acc ttc aGc

1-e cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag
gtc tcc tgc aag gct tct gga GGc acc ttc aGc

1-f Gag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcT Aca gtg aaA
Atc tcc tgc aag gTt tct gga tac acc ttc acc

VH2

2-05 CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACA CAG ACC CTC ACG
CTG ACC TGC ACC TTC TCT GGG TTC TCA CTC AGC

2-26 cag Gtc acc ttg aag gag tct ggt cct GTg ctg gtg aaa ccc aca Gag acc ctc acg
ctg acc tgc acc Gtc tct ggg ttc tca ctc agc

2-70 cag Gtc acc ttg aag gag tct ggt cct Gcg ctg gtg aaa ccc aca cag acc ctc acA
ctg acc tgc acc ttc tct ggg ttc tca ctc agc

VH3

3-07 GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGG GGG TCC CTG AGA
CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGT

3-09 gaA gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggC Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt GAt

3-11 Cag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc Aag cct ggA ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-13 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-15 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA Aag cct ggg ggg tcc ctT aga
ctc tcc tgt gca gcc tct gga ttc acT ttC agt

3-20 gag gtg cag ctg gtg gag tct ggg gga ggT Gtg gtA cGg cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt GAt
3-21 gag gtg cag ctg gtg gag tct ggg gga ggc Ctg gtc Aag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-23 gag gtg cag ctg Ttg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt agC
3-30 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-30.3 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-30.5 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-33 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcG tct gga ttc acc ttC agt
3-43 gaA gtg cag ctg gtg gag tct ggg gga gTc Gtg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt GAt
3-48 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-49 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag ccA ggg Cgg tcc ctg aga
ctc tcc tgt Aca gcT tct gga ttc acc ttt Ggt
3-53 gag gtg cag ctg gtg gag Act ggA gga ggc ttg Atc cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct ggG ttc acc GtC agt
3-64 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-66 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc GtC agt
3-72 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggA ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-73 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aAa
ctc tcc tgt gca gcc tct ggG ttc acc ttC agt
3-74 gag gtg cag ctg gtg gag tcC ggg gga ggc ttA gtT cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt
3-d gag gtg cag ctg gtg gag tct Cgg gga gTc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc GtC agt

VH4

4-04 CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GGG ACC CTG TCC
CTC ACC TGC GCT GTC TCT GGT GGC TCC ATC AGC
4-28 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAC acc ctg tcc
ctc acc tgc gct gtc tct ggt TAc tcc atc agc
4-30.1 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc
4-30.2 cag Ctg cag ctg cag gag tcC ggc Tca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc gct gtc tct ggt ggc tcc atc agc

4-30.4 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-31 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-34 cag gtg cag ctA cag Cag tGg ggc Gca gga ctg Ttg aag cct tcg gAg acc ctg tcc
ctc acc tgc gct gtc tAt ggt ggG tcc Ttc agT

4-39 cag Ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-59 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agT

4-61 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc Gtc agc

4-b cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc gct gtc tct ggt TAc tcc atc agc

VH5

5-51 GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AAG
ATC TCC TGT AAG GGT TCT GGA TAC AGC TTT ACC

5-a gaA gtg cag ctg gtg cag tct gga gca gag gtg aaa aag ccc ggg gag tct ctg aGg
atc tcc tgt aag ggt tct gga tac agc ttt acc

VH6

6-1 CAG GTA CAG CTG CAG CAG TCA GGT CCA GGA CTG GTG AAG CCC TCG CAG ACC CTC TCA
CTC ACC TGT GCC ATC TCC GGG GAC AGT GTC TCT

VH7

7-4.1 CAG GTG CAG CTG GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC TCA GTG AAG
GTT TCC TGC AAG GCT TCT GGA TAC ACC TTC ACT

Table 7: RERS sites in Human HC GLG FR1s where there are at least 20 GLGs cut

BsgI GTGCAG 71 (cuts 16/14 bases to right)

1: 4 1: 13 2: 13 3: 4 3: 13 4: 13
6: 13 7: 4 7: 13 8: 13 9: 4 9: 13
10: 4 10: 13 15: 4 15: 65 16: 4 16: 65
17: 4 17: 65 18: 4 18: 65 19: 4 19: 65
20: 4 20: 65 21: 4 21: 65 22: 4 22: 65
23: 4 23: 65 24: 4 24: 65 25: 4 25: 65
26: 4 26: 65 27: 4 27: 65 28: 4 28: 65
29: 4 30: 4 30: 65 31: 4 31: 65 32: 4
32: 65 33: 4 33: 65 34: 4 34: 65 35: 4
35: 65 36: 4 36: 65 37: 4 38: 4 39: 4
41: 4 42: 4 43: 4 45: 4 46: 4 47: 4
48: 4 48: 13 49: 4 49: 13 51: 4

There are 39 hits at base# 4

There are 21 hits at base# 65

-"- ctgcac 9

12: 63 13: 63 14: 63 39: 63 41: 63 42: 63
44: 63 45: 63 46: 63

BbvI GCAGC 65

1: 6 3: 6 6: 6 7: 6 8: 6 9: 6
10: 6 15: 6 15: 67 16: 6 16: 67 17: 6
17: 67 18: 6 18: 67 19: 6 19: 67 20: 6
20: 67 21: 6 21: 67 22: 6 22: 67 23: 6
23: 67 24: 6 24: 67 25: 6 25: 67 26: 6
26: 67 27: 6 27: 67 28: 6 28: 67 29: 6
30: 6 30: 67 31: 6 31: 67 32: 6 32: 67
33: 6 33: 67 34: 6 34: 67 35: 6 35: 67
36: 6 36: 67 **37:** 6 **38:** 6 **39:** 6 **40:** 6
41: 6 **42:** 6 43: 6 **44:** 6 **45:** 6 **46:** 6
47: 6 48: 6 49: 6 **50:** 12 51: 6

There are 43 hits at base# 6 Bolded sites very near sites listed below

There are 21 hits at base# 67

-"- gctgc 13

37: 9 **38:** 9 **39:** 9 **40:** 3 **40:** 9 **41:** 9
42: 9 **44:** 3 **44:** 9 **45:** 9 **46:** 9 **47:** 9

50: 9

There are 11 hits at base# 9

BsoFI GCngc 78

1: 6 3: 6 6: 6 7: 6 8: 6 9: 6
10: 6 15: 6 15: 67 16: 6 16: 67 17: 6
17: 67 18: 6 18: 67 19: 6 19: 67 20: 6
20: 67 21: 6 21: 67 22: 6 22: 67 23: 6
23: 67 24: 6 24: 67 25: 6 25: 67 26: 6
26: 67 27: 6 27: 67 28: 6 28: 67 29: 6
30: 6 30: 67 31: 6 31: 67 32: 6 32: 67
33: 6 33: 67 34: 6 34: 67 35: 6 35: 67
36: 6 36: 67 37: 6 37: 9 38: 6 38: 9
39: 6 39: 9 40: 3 40: 6 40: 9 41: 6
41: 9 42: 6 42: 9 43: 6 44: 3 44: 6
44: 9 45: 6 45: 9 46: 6 46: 9 47: 6
47: 9 48: 6 49: 6 50: 9 50: 12 51: 6

There are 43 hits at base# 6 These often occur together.

There are 11 hits at base# 9

There are 2 hits at base# 3

There are 21 hits at base# 67

TseI Gcwgc 78

1: 6 3: 6 6: 6 7: 6 8: 6 9: 6
10: 6 15: 6 15: 67 16: 6 16: 67 17: 6
17: 67 18: 6 18: 67 19: 6 19: 67 20: 6
20: 67 21: 6 21: 67 22: 6 22: 67 23: 6
23: 67 24: 6 24: 67 25: 6 25: 67 26: 6
26: 67 27: 6 27: 67 28: 6 28: 67 29: 6
30: 6 30: 67 31: 6 31: 67 32: 6 32: 67
33: 6 33: 67 34: 6 34: 67 35: 6 35: 67
36: 6 36: 67 37: 6 37: 9 38: 6 38: 9
39: 6 39: 9 40: 3 40: 6 40: 9 41: 6
41: 9 42: 6 42: 9 43: 6 44: 3 44: 6
44: 9 45: 6 45: 9 46: 6 46: 9 47: 6
47: 9 48: 6 49: 6 50: 9 50: 12 51: 6

There are 43 hits at base# 6 Often together.

There are 11 hits at base# 9

There are 2 hits at base# 3

There are 1 hits at base# 12

There are 21 hits at base# 67

MspA1I CMGckg 48

1: 7	3: 7	4: 7	5: 7	6: 7	7: 7
8: 7	9: 7	10: 7	11: 7	15: 7	16: 7
17: 7	18: 7	19: 7	20: 7	21: 7	22: 7
23: 7	24: 7	25: 7	26: 7	27: 7	28: 7
29: 7	30: 7	31: 7	32: 7	33: 7	34: 7
35: 7	36: 7	37: 7	38: 7	39: 7	40: 1
40: 7	41: 7	42: 7	44: 1	44: 7	45: 7
46: 7	47: 7	48: 7	49: 7	50: 7	51: 7

There are 46 hits at base# 7

PvuII CAGctg 48

1: 7	**3: 7**	**4: 7**	**5: 7**	**6: 7**	**7: 7**
8: 7	**9: 7**	**10: 7**	**11: 7**	**15: 7**	**16: 7**
17: 7	**18: 7**	**19: 7**	**20: 7**	**21: 7**	**22: 7**
23: 7	**24: 7**	**25: 7**	**26: 7**	**27: 7**	**28: 7**
29: 7	**30: 7**	**31: 7**	**32: 7**	**33: 7**	**34: 7**
35: 7	**36: 7**	**37: 7**	**38: 7**	**39: 7**	**40: 1**
40: 7	**41: 7**	**42: 7**	**44: 1**	**44: 7**	**45: 7**
46: 7	**47: 7**	**48: 7**	**49: 7**	**50: 7**	**51: 7**

There are 46 hits at base# 7

There are 2 hits at base# 1

AluI AGct 54

1: 8	2: 8	3: 8	4: 8	4: 24	5: 8
6: 8	7: 8	8: 8	9: 8	10: 8	11: 8
15: 8	16: 8	17: 8	18: 8	19: 8	20: 8
21: 8	22: 8	23: 8	24: 8	25: 8	26: 8
27: 8	28: 8	29: 8	29: 69	30: 8	31: 8
32: 8	33: 8	34: 8	35: 8	36: 8	37: 8
38: 8	39: 8	40: 2	40: 8	41: 8	42: 8
43: 8	44: 2	44: 8	45: 8	46: 8	47: 8
48: 8	48: 82	49: 8	49: 82	50: 8	51: 8

There are 48 hits at base# 8

There are 2 hits at base# 2

DdeI Ctnag 48

1: 26 1: 48 2: 26 2: 48 3: 26 3: 48
4: 26 4: 48 5: 26 5: 48 6: 26 6: 48
7: 26 7: 48 8: 26 8: 48 9: 26 10: 26
11: 26 12: 85 13: 85 14: 85 15: 52 16: 52
17: 52 18: 52 19: 52 20: 52 21: 52 22: 52
23: 52 24: 52 25: 52 26: 52 27: 52 28: 52
29: 52 30: 52 31: 52 32: 52 33: 52 35: 30
35: 52 36: 52 40: 24 49: 52 51: 26 51: 48

There are 22 hits at base# 52 52 and 48 never together.

There are 9 hits at base# 48

There are 12 hits at base# 26 26 and 24 never together.

HphI tcacc 42

1: 86 3: 86 6: 86 7: 86 8: 80 11: 86
12: 5 13: 5 14: 5 15: 80 16: 80 17: 80
18: 80 20: 80 21: 80 22: 80 23: 80 24: 80
25: 80 26: 80 27: 80 28: 80 29: 80 30: 80
31: 80 32: 80 33: 80 34: 80 35: 80 36: 80
37: 59 38: 59 39: 59 40: 59 41: 59 42: 59
43: 59 44: 59 45: 59 46: 59 47: 59 50: 59

There are 22 hits at base# 80 80 and 86 never together

There are 5 hits at base# 86

There are 12 hits at base# 59

BssKI Nccngg 50

1: 39 2: 39 3: 39 4: 39 5: 39 7: 39
8: 39 9: 39 10: 39 11: 39 15: 39 16: 39
17: 39 18: 39 19: 39 20: 39 21: 29 21: 39
22: 39 23: 39 24: 39 25: 39 26: 39 27: 39
28: 39 29: 39 30: 39 31: 39 32: 39 33: 39
34: 39 35: 19 35: 39 36: 39 37: 24 38: 24
39: 24 41: 24 42: 24 44: 24 45: 24 46: 24
47: 24 48: 39 48: 40 49: 39 49: 40 50: 24
50: 73 51: 39

There are 35 hits at base# 39 39 and 40 together twice.
There are 2 hits at base# 40

BsaJI Ccnngg 47

1: 40 2: 40 3: 40 4: 40 5: 40 7: 40
8: 40 9: 40 9: 47 10: 40 10: 47 11: 40
15: 40 18: 40 19: 40 20: 40 21: 40 22: 40
23: 40 24: 40 25: 40 26: 40 27: 40 28: 40
29: 40 30: 40 31: 40 32: 40 34: 40 35: 20
35: 40 36: 40 37: 24 38: 24 39: 24 41: 24
42: 24 44: 24 45: 24 46: 24 47: 24 48: 40
48: 41 49: 40 49: 41 50: 74 51: 40

There are 32 hits at base# 40 40 and 41 together twice
There are 2 hits at base# 41
There are 9 hits at base# 24
There are 2 hits at base# 47

BstNI CCwgg 44
PspGI ccwgg
ScrFI($M.HpaII) CCwgg
1: 40 2: 40 3: 40 4: 40 5: 40 7: 40
8: 40 9: 40 10: 40 11: 40 15: 40 16: 40
17: 40 18: 40 19: 40 20: 40 21: 30 21: 40
22: 40 23: 40 24: 40 25: 40 26: 40 27: 40
28: 40 29: 40 30: 40 31: 40 32: 40 33: 40
34: 40 35: 40 36: 40 37: 25 38: 25 39: 25
41: 25 42: 25 44: 25 45: 25 46: 25 47: 25
50: 25 51: 40

There are 33 hits at base# 40

ScrFI CCngg 50

1: 40 2: 40 3: 40 4: 40 5: 40 7: 40
8: 40 9: 40 10: 40 11: 40 15: 40 16: 40
17: 40 18: 40 19: 40 20: 40 21: 30 21: 40
22: 40 23: 40 24: 40 25: 40 26: 40 27: 40
28: 40 29: 40 30: 40 31: 40 32: 40 33: 40
34: 40 35: 20 35: 40 36: 40 37: 25 38: 25
39: 25 41: 25 42: 25 44: 25 45: 25 46: 25

47: 25 48: 40 48: 41 49: 40 49: 41 50: 25
50: 74 51: 40
There are 35 hits at base# 40
There are 2 hits at base# 41

EcoO109I RGgnccy 34
1: 43 2: 43 3: 43 4: 43 5: 43 6: 43
7: 43 8: 43 9: 43 10: 43 15: 46 16: 46
17: 46 18: 46 19: 46 20: 46 21: 46 22: 46
23: 46 24: 46 25: 46 26: 46 27: 46 28: 46
30: 46 31: 46 32: 46 33: 46 34: 46 35: 46
36: 46 37: 46 43: 79 51: 43
There are 22 hits at base# 46 46 and 43 never together
There are 11 hits at base# 43

NlaIV GGNncc 71
1: 43 2: 43 3: 43 4: 43 5: 43 6: 43
7: 43 8: 43 9: 43 9: 79 10: 43 10: 79
15: 46 15: 47 16: 47 17: 46 17: 47 18: 46
18: 47 19: 46 19: 47 20: 46 20: 47 21: 46
21: 47 22: 46 22: 47 23: 47 24: 47 25: 47
26: 47 27: 46 27: 47 28: 46 28: 47 29: 47
30: 46 30: 47 31: 46 31: 47 32: 46 32: 47
33: 46 33: 47 34: 46 34: 47 35: 46 35: 47
36: 46 36: 47 37: 21 37: 46 37: 47 37: 79
38: 21 39: 21 39: 79 40: 79 41: 21 41: 79
42: 21 42: 79 43: 79 44: 21 44: 79 45: 21
45: 79 46: 21 46: 79 47: 21 51: 43
There are 23 hits at base# 47 46 & 47 often together
There are 17 hits at base# 46 There are 11 hits at base# 43

Sau96I Ggncc 70
1: 44 2: 3 2: 44 3: 44 4: 44 5: 3 5: 44 6: 44
7: 44 8: 22 8: 44 9: 44 10: 44 11: 3 12: 22 13: 22
14: 22 15: 33 15: 47 16: 47 17: 47 18: 47 19: 47 20: 47
21: 47 22: 47 23: 33 23: 47 24: 33 24: 47 25: 33 25: 47
26: 33 26: 47 27: 47 28: 47 29: 47 30: 47 31: 33 31: 47
32: 33 32: 47 33: 33 33: 47 34: 33 34: 47 35: 47 36: 47
37: 21 37: 22 37: 47 38: 21 38: 22 39: 21 39: 22 41: 21
41: 22 42: 21 42: 22 43: 80 44: 21 44: 22 45: 21 45: 22

46: 21 46: 22 47: 21 47: 22 50: 22 51: 44
There are 23 hits at base# 47 These do not occur together.
There are 11 hits at base# 44
There are 14 hits at base# 22 These do occur together.
There are 9 hits at base# 21

BsmAI GTCTCNnnnn 22
1: 58 3: 58 4: 58 5: 58 8: 58 9: 58
10: 58 13: 70 36: 18 37: 70 38: 70 39: 70
40: 70 41: 70 42: 70 44: 70 45: 70 46: 70
47: 70 48: 48 49: 48 50: 85
There are 11 hits at base# 70

-"- Nnnnnngagac 27
13: 40 15: 48 16: 48 17: 48 18: 48 20: 48
21: 48 22: 48 23: 48 24: 48 25: 48 26: 48
27: 48 28: 48 29: 48 30: 10 30: 48 31: 48
32: 48 33: 48 35: 48 36: 48 43: 40 44: 40
45: 40 46: 40 47: 40
There are 20 hits at base# 48

AvaII Ggwcc 44
Sau96I($M.HaeIII) Ggwcc 44
2: 3 5: 3 6: 44 8: 44 9: 44 10: 44
11: 3 12: 22 13: 22 14: 22 15: 33 15: 47
16: 47 17: 47 18: 47 19: 47 20: 47 21: 47
22: 47 23: 33 23: 47 24: 33 24: 47 25: 33
25: 47 26: 33 26: 47 27: 47 28: 47 29: 47
30: 47 31: 33 31: 47 32: 33 32: 47 33: 33
33: 47 34: 33 34: 47 35: 47 36: 47 37: 47
43: 80 50: 22
There are 23 hits at base# 47 44 & 47 never together
There are 4 hits at base# 44

PpuMI RGgwccy 27
6: 43 8: 43 9: 43 10: 43 15: 46 16: 46
17: 46 18: 46 19: 46 20: 46 21: 46 22: 46
23: 46 24: 46 25: 46 26: 46 27: 46 28: 46

30: 46 31: 46 32: 46 33: 46 34: 46 35: 46
36: 46 37: 46 43: 79

There are 22 hits at base# 46 43 and 46 never occur together.

There are 4 hits at base# 43

BsmFI GGGAC 3

8: 43 37: 46 50: 77

-"- gtccc 33

15: 48 16: 48 17: 48 1: 0 1: 0 20: 48
21: 48 22: 48 23: 48 24: 48 25: 48 26: 48
27: 48 28: 48 29: 48 30: 48 31: 48 32: 48
33: 48 34: 48 35: 48 36: 48 37: 54 38: 54
39: 54 40: 54 41: 54 42: 54 43: 54 44: 54
45: 54 46: 54 47: 54

There are 20 hits at base# 48

There are 11 hits at base# 54

HinfI Gantc 80

8: 77 12: 16 13: 16 14: 16 15: 16 15: 56
15: 77 16: 16 16: 56 16: 77 17: 16 17: 56
17: 77 18: 16 18: 56 18: 77 19: 16 19: 56
19: 77 20: 16 20: 56 20: 77 21: 16 21: 56
21: 77 22: 16 22: 56 22: 77 23: 16 23: 56
23: 77 24: 16 24: 56 24: 77 25: 16 25: 56
25: 77 26: 16 26: 56 26: 77 27: 16 27: 26
27: 56 27: 77 28: 16 28: 56 28: 77 29: 16
29: 56 29: 77 30: 56 31: 16 31: 56 31: 77
32: 16 32: 56 32: 77 33: 16 33: 56 33: 77
34: 16 35: 16 35: 56 35: 77 36: 16 36: 26
36: 56 36: 77 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16
48: 46 49: 46

There are 34 hits at base# 16

TfiI Gawtc 21

8: 77 15: 77 16: 77 17: 77 18: 77 19: 77
20: 77 21: 77 22: 77 23: 77 24: 77 25: 77

26: 77 27: 77 28: 77 29: 77 31: 77 32: 77
33: 77 35: 77 36: 77
There are 21 hits at base# 77

MlyI GAGTC 38
12: 16 13: 16 14: 16 15: 16 16: 16 17: 16
18: 16 19: 16 20: 16 21: 16 22: 16 23: 16
24: 16 25: 16 26: 16 27: 16 27: 26 28: 16
29: 16 31: 16 32: 16 33: 16 34: 16 35: 16
36: 16 36: 26 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16
48: 46 49: 46
There are 34 hits at base# 16

-"- GACTC 21
15: 56 16: 56 17: 56 18: 56 19: 56 20: 56
21: 56 22: 56 23: 56 24: 56 25: 56 26: 56
27: 56 28: 56 29: 56 30: 56 31: 56 32: 56
33: 56 35: 56 36: 56
There are 21 hits at base# 56

PleI gagtc 38
12: 16 13: 16 14: 16 15: 16 16: 16 17: 16
18: 16 19: 16 20: 16 21: 16 22: 16 23: 16
24: 16 25: 16 26: 16 27: 16 27: 26 28: 16
29: 16 31: 16 32: 16 33: 16 34: 16 35: 16
36: 16 36: 26 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16
48: 46 49: 46
There are 34 hits at base# 16

-"- gactc 21
15: 56 16: 56 17: 56 18: 56 19: 56 20: 56
21: 56 22: 56 23: 56 24: 56 25: 56 26: 56
27: 56 28: 56 29: 56 30: 56 31: 56 32: 56
33: 56 35: 56 36: 56
There are 21 hits at base# 56

AlwNI CAGNNNctg 26
15: 68 16: 68 17: 68 18: 68 19: 68 20: 68

21: 68	22: 68	23: 68	24: 68	25: 68	26: 68
27: 68	28: 68	29: 68	30: 68	31: 68	32: 68
33: 68	34: 68	35: 68	36: 68	39: 46	40: 46
41: 46	42: 46				

There are 22 hits at base# 68

Table 8: Kappa FR1 GLGs

```
! 1   2   3   4   5   6   7   8   9   10  11  12
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
! 13  14  15  16  17  18  19  20  21  22  23
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     O12
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     O2
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     O18
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     O8
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     A20
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     A30
  AAC ATC CAG ATG ACC CAG TCT CCA TCT GCC ATG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !     L14
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !     L1
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !     L15
  GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     L4
  GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     L18
  GAC ATC CAG ATG ACC CAG TCT CCA TCT TCC GTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !     L5
  GAC ATC CAG ATG ACC CAG TCT CCA TCT TCT GTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !     L19
  GAC ATC CAG TTG ACC CAG TCT CCA TCC TTC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     L8
  GCC ATC CGG ATG ACC CAG TCT CCA TTC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !     L23
  GCC ATC CGG ATG ACC CAG TCT CCA TCC TCA TTC TCT
  GCA TCT ACA GGA GAC AGA GTC ACC ATC ACT TGT  !     L9
```

GTC ATC TGG ATG ACC CAG TCT CCA TCC TTA CTC TCT
GCA TCT ACA GGA GAC AGA GTC ACC ATC AGT TGT ! L24
GCC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L11
GAC ATC CAG ATG ACC CAG TCT CCT TCC ACC CTG TCT
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L12
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O11
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O1
GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A17
GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A1
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A18
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A2
GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A19
GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A3
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC TCA CCT
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A23
GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A27
GAA ATT GTG TTG ACG CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A11
GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L2
GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L16
GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L6
GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT

TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L20
GAA ATT GTA ATG ACA CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L25
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT
GTG TCT CTG GGC GAG AGG GCC ACC ATC AAC TGC ! B3
GAA ACG ACA CTC ACG CAG TCT CCA GCA TTC ATG TCA
GCG ACT CCA GGA GAC AAA GTC AAC ATC TCC TGC ! B2
GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT
GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC ! A26
GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT
GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC ! A10
GAT GTT GTG ATG ACA CAG TCT CCA GCT TTC CTC TCT
GTG ACT CCA GGG GAG AAA GTC ACC ATC ACC TGC ! A14

Table 9 RERS sites found in Human Kappa FR1 GLGs

	MslI	FokI --> <-- -->	PflFI	BsrI	BsmAI	MnlI	HpyCH 4V
VK1							
O12 1-69	3	3 23	12 49	15	18 47	26	36
O2 101-169	103	103 123	112 149	115	118 147	126	136
O18 201-269	203	203 223	212 249	215	218 247	226	236
O8 301-369	303	303 323	312 349	315	318 347	326	336
A20 401-469	403	403 423	412 449	415	418 447	426	436
A30 501-569	503	503 523	512 549	515	518 547	526	536
L14 601-669	603	603	612 649	615	618 647	-	636
L1 701-769	703	703 723	712 749	715	718 747	726	736
L15 801-869	803	803 823	812 849	815	818 847	826	836
L4 901-969	-	903 923	912 949	906 915	918 947	926	936
L18 1001-1069	-	1003	1012 1049	1006 1015	1018 1047	1026	1036
L5 1101-1169	1103	-	1112 1149	1115	1118 1147	-	1136
L19 1201-1269	1203	1203	1212 1249	1215	1218 1247	-	1236
L8 1301-1369	-	1303 1323	1312 1349	1306 1315	1318 1347	-	1336
L23 1401-1469	1403	1403 1408	1412 1449	1415	1418 1447	-	1436
L9 1501-1569	1503	1503 1508 1523	1512 1549	1515	1518 1547	1526	1536

	MslI	FokI --> <-- -->	PflFI	BsrI	BsmAI	MnlI	HpyCH 4V
L24 1601-1669	1603	1608 1623	1612 1649	1615	1618 1647	-	1636
L11 1701-1769	1703	1703 1723	1712 1749	1715	1718 1747	1726	1736
L12 1801-1869	1803	1803	1812 1849	1815	1818 1847	-	1836
VKII							
O11 1901-1969	-	-	-	-	-	1956	-
O1 2001-2069	-	-	-	-		2056	-
A17 2101-2169	-	-	2112	-	2118	2156	-
A1 2201-2269	-	-	2212	-	2218	2256	-
A18 2301-2369	-	-	-	-	-	2356	-
A2 2401-2469	-	-	-	-	-	2456	-
A19 2501-2569	-	-	2512	-	2518	2556	-
A3 2601-2669	-	-	2612	-	2618	2656	-
A23 2701-2769	-	-	-	-	-	2729 2756	-
VKIII							
A27 2801-2869	-	-	2812	-	2818 2839	2860	-
A11 2901-2969	-	-	2912	-	2918 2939	2960	-
L2 3001-3069	-	-	3012	-	3018 3039	3060	-
L16 3101-3169	-	-	3112	-	3118 3139	3160	-

	MslI	FokI --> <-- -->	PflFI	BsrI	BsmAI	MnlI	HpyCH 4V
L6 3201-3269	-	-	3212	-	3218 3239	3260	-
L20 3301-3369	-	-	3312	-	3318 3339	3360	-
L25 3401-3469	-	-	3412	-	3418 3439	3460	-
VKIV							
B3 3501-3569	3503	-	3512	3515	3518 3539	3551<	-
VKV							
B2 3601-3669	-	-	3649	-	3618 3647		-
VKVI							
A26 3701-3769	-	-	3712	-	3718		-
A10 3801-3869	-	-	3812	-	3818		-
A14 3901-3969	-	-	3912	-	3918	3930>	-

Table 9 RERS sites found in Human Kappa FR1 GLGs, continued

	SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
VKI							
O12 1-69	37	41	53	53	55	56	-
O2 101-169	137	141	153	153	155	156	-
O18 201-269	237	241	253	253	255	256	-
O8 301-369	337	341	353	353	355	356	-
A20 401-469	437	441	453	453	455	456	-
A30 501-569	537	541	553	553	555	556	-
L14 601-669	637	641	653	653	655	656	-
L1 701-769	737	741	753	753	755	756	-
L15 801-869	837	841	853	853	855	856	-
L4 901-969	937	941	953	953	955	956	-
L18 1001-1069	1037	1041	1053	1053	1055	1056	-
L5 1101-1169	1137	1141	1153	1153	1155	1156	-
L19 1201-1269	1237	1241	1253	1253	1255	1256	-
L8 1301-1369	1337	1341	1353	1353	1355	1356	-
L23 1401-1469	1437	1441	1453	1453	1455	1456	1406

	SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
L9 1501-1569	1537	1541	1553	1553	1555	1556	1506
L24 1601-1669	1637	1641	1653	1653	1655	1656	
L11 1701-1769	1737	1741	1753	1753	1755	1756	
L12 1801-1869	1837	1841	1853	1853	1855	1856	
VKII							
O11 1901-1969	-	-	1918	1918	1937	1938	1952
O1 2001-2069	-	-	2018	2018	2037	2038	2052
A17 2101-2169	-	-	2112	2112	2137	2138	2152
A1 2201-2269	-	-	2212	2212	2237	2238	2252
A18 2301-2369	-	-	2318	2318	2337	2338	2352
A2 2401-2469	-	-	2418	2418	2437	2438	2452
A19 2501-2569	-	-	2512	2512	2537	2538	2552
A3 2601-2669	-	-	2612	2612	2637	2638	2652
A23 2701-2769	-	-	2718	2718	2737	2731* 2738*	-
VKIII							
A27 2801-2869	-	-	-	-			-
A11 2901-2969	-	-	-	-			-

	SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
L2 3001-3069	-	-	-	-			-
L16 3101-3169	-	-	-	-			-
L6 3201-3269	-	-	-	-			-
L20 3301-3369	-	-	-	-			-
L25 3401-3469	-	-	-	-			-
VKIV							
B3 3501-3569	-	-	3525	3525			-
VKV							
B2 3601-3669	-	-	3639	3639			-
VKVI							
A26 3701-3769	-	-	3712 3739	3712 3739	3737 3755	3756 3762	-
A10 3801-3869	-	-	3812 3839	3812 3839	3837 3855	3856 3862	-
A14 3901-3969	-	-	3939	3939	3937 3955	3956 3962	-

Table 9 RERS sites found in Human Kappa FR1, continued

	BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMIV	HaeIII	Tsp509I
VKI						
O12 1-69	-	-	-	-	-	-
O2 101-169	-	-	-	-	-	-
O18 201-269	-	-	-	-	-	-
O8 301-369	-	-	-	-	-	-
A20 401-469	-	-	-	-	-	-
A30 501-569	-	-	-	-	-	-
L14 601-669	-	-	-	-	-	-
L1 701-769	-	-	-	-	-	-
L15 801-869	-	-	-	-	-	-
L4 901-969	-	-	-	-	-	-
L18 1001-1069	-	-	-	-	-	-
L5 1101-1169	-	-	-	-	-	-
L19 1201-1269	-	-	-	-	-	-
L8 1301-1369	-	-	-	-	-	-

	BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMIV	HaeIII	Tsp509I
L23 1401-1469	-	-	-	-	-	-
L9 1501-1569	-	-	-	-	-	-
L24 1601-1669	-	-	-	-	-	-
L11 1701-1769	-	-	-	-	-	-
L12 1801-1869	-	-	-	-	-	-
VKII						
O11 1901-1969	1942	1943	1944	1951	1954	-
O1 2001-2069	2042	2043	2044	2051	2054	-
A17 2101-2169	2142	-	-	2151	2154	-
A1 2201-2269	2242	-	-	2251	2254	-
A18 2301-2369	2342	2343	-	2351	2354	-
A2 2401-2469	2442	2443	-	2451	2454	-
A19 2501-2569	2542	2543	2544	2551	2554	-
A3 2601-2669	2642	2643	2644	2651	2654	-
A23 2701-2769	2742	-	-	2751	2754	-
VKIII						

	BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMIV	HaeIII	Tsp509I
A27 2801-2869	2843	2822 2843	2820 2841	-	-	2803
A11 2901-2969	2943	2943	2920 2941	-	-	2903
L2 3001-3069	3043	3043	3041	-	-	-
L16 3101-3169	3143	3143	3120 3141	-	-	-
L6 3201-3269	3243	3243	3220 3241	-	-	3203
L20 3301-3369	3343	3343	3320 3341	-	-	3303
L25 3401-3469	3443	3443	3420 3441	-	-	3403
VKIV						
B3 3501-3569	3529	3530	3520	-	3554	
VKV						
B2 3601-3669		3643	3620 3641	-	-	
VKVI						
A26 3701-3769		-	3720	-	-	3703
A10 3801-3869		-	3820	-	-	3803
A14 3901-3969	3943	3943	3920 3941	-	-	-

Table 10 Lambda FR1 GLG sequences

```
! VL1
            CAG TCT GTG CTG ACT CAG CCA CCC TCG GTG TCT GAA
            GCC CCC AGG CAG AGG GTC ACC ATC TCC TGT !  1a
 5          cag tct gtg ctg acG cag ccG ccc tcA gtg tct gGG
            gcc ccA Ggg cag agg gtc acc atc tcc tgC !  1e
            cag tct gtg ctg act cag cca ccc tcA gCg tct gGG
            Acc ccc Ggg cag agg gtc acc atc tcT tgt !  1c
            cag tct gtg ctg act cag cca ccc tcA gCg tct gGG
10          Acc ccc Ggg cag agg gtc acc atc tcT tgt !  1g
            cag tct gtg Ttg acG cag ccG ccc tcA gtg tct gCG
            gcc ccA GgA cag aAg gtc acc atc tcc tgC !  1b
! VL2
            CAG TCT GCC CTG ACT CAG CCT CCC TCC GCG TCC GGG
15          TCT CCT GGA CAG TCA GTC ACC ATC TCC TGC !  2c
            cag tct gcc ctg act cag cct cGc tcA gTg tcc ggg
            tct cct gga cag tca gtc acc atc tcc tgc!  2e
            cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg
            tct cct gga cag tcG Atc acc atc tcc tgc !  2a2
20          cag tct gcc ctg act cag cct ccc tcc gTg tcc ggg
            tct cct gga cag tca gtc acc atc tcc tgc !  2d
            cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg
            tct cct gga cag tcG Atc acc atc tcc tgc !  2b2
! VL3
25          TCC TAT GAG CTG ACT CAG CCA CCC TCA GTG TCC GTG
            TCC CCA GGA CAG ACA GCC AGC ATC ACC TGC!   3r
            tcc tat gag ctg act cag cca cTc tca gtg tcA gtg
            Gcc cTG gga cag acG gcc agG atT acc tgT !  3j
            tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg
30          tcc cca gga caA acG gcc agG atc acc tgc!  3p
            tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg
            tcc cTa gga cag aTG gcc agG atc acc tgc !  3a
            tcT tCt gag ctg act cag GAC ccT GcT gtg tcT gtg
            Gcc TTG gga cag aca gTc agG atc acA tgc !  3l
```

tcc tat gTg ctg act cag cca ccc tca gtg tcA gtg
Gcc cca gga Aag acG gcc agG atT acc tgT ! 3h
tcc tat gag ctg acA cag cTa ccc tcG gtg tcA gtg
tcc cca gga cag aca gcc agG atc acc tgc ! 3e
tcc tat gag ctg aTG cag cca ccc tcG gtg tcA gtg
tcc cca gga cag acG gcc agG atc acc tgc ! 3m
tcc tat gag ctg acA cag cca Tcc tca gtg tcA gtg
tcT ccG gga cag aca gcc agG atc acc tgc ! V2-19

! VL4

CTG CCT GTG CTG ACT CAG CCC CCG TCT GCA TCT GCC
TTG CTG GGA GCC TCG ATC AAG CTC ACC TGC ! 4c
cAg cct gtg ctg act caA TcA TcC tct gcC tct gcT
tCC ctg gga Tcc tcg Gtc aag ctc acc tgc ! 4a
cAg cTt gtg ctg act caA TcG ccC tct gcC tct gcc
tCC ctg gga gcc tcg Gtc aag ctc acc tgc ! 4b

! VL5

CAG CCT GTG CTG ACT CAG CCA CCT TCC TCC TCC GCA
TCT CCT GGA GAA TCC GCC AGA CTC ACC TGC ! 5e
cag Gct gtg ctg act cag ccG Gct tcc CTc tcT gca
tct cct gga gCa tcA gcc agT ctc acc tgc ! 5c
cag cct gtg ctg act cag cca Tct tcc CAT tcT gca
tct Tct gga gCa tcA gTc aga ctc acc tgc ! 5b

! VL6

AAT TTT ATG CTG ACT CAG CCC CAC TCT GTG TCG GAG
TCT CCG GGG AAG ACG GTA ACC ATC TCC TGC ! 6a

! VL7

CAG ACT GTG GTG ACT CAG GAG CCC TCA CTG ACT GTG
TCC CCA GGA GGG ACA GTC ACT CTC ACC TGT ! 7a
cag Gct gtg gtg act cag gag ccc tca ctg act gtg
tcc cca gga ggg aca gtc act ctc acc tgt ! 7b

! VL8

CAG ACT GTG GTG ACC CAG GAG CCA TCG TTC TCA GTG
TCC CCT GGA GGG ACA GTC ACA CTC ACT TGT ! 8a

! VL9

CAG CCT GTG CTG ACT CAG CCA CCT TCT GCA TCA GCC
TCC CTG GGA GCC TCG GTC ACA CTC ACC TGC ! 9a

! VL10

CAG GCA GGG CTG ACT CAG CCA CCC TCG GTG TCC AAG
GGC TTG AGA CAG ACC GCC ACA CTC ACC TGC ! 10a

Table 11 RERSs found in human lambda FR1 GLGs

```
! There are 31 lambda GLGs
MlyI NnnnnnGACTC                 25
  1:  6     3:  6     4:  6     6:  6     7:  6     8:  6
  9:  6    10:  6    11:  6    12:  6    15:  6    16:  6
 20:  6    21:  6    22:  6    23:  6    23: 50    24:  6
 25:  6    25: 50    26:  6    27:  6    28:  6    30:  6
 31:  6
 There are  23 hits at base#  6

-"-  GAGTCNNNNNn                 1
 26: 34

MwoI GCNNNNNnngc                 20
  1:  9     2:  9     3:  9     4:  9    11:  9    11: 56
 12:  9    13:  9    14:  9    16:  9    17:  9    18:  9
 19:  9    20:  9    23:  9    24:  9    25:  9    26:  9
 30:  9    31:  9
 There are  19 hits at base#  9
HinfI Gantc                      27
  1: 12     3: 12     4: 12     6: 12     7: 12     8: 12
  9: 12    10: 12    11: 12    12: 12    15: 12    16: 12
 20: 12    21: 12    22: 12    23: 12    23: 46    23: 56
 24: 12    25: 12    25: 56    26: 12    26: 34    27: 12
 28: 12    30: 12    31: 12
 There are  23 hits at base# 12
PleI gactc                       25
  1: 12     3: 12     4: 12     6: 12     7: 12     8: 12
  9: 12    10: 12    11: 12    12: 12    15: 12    16: 12
 20: 12    21: 12    22: 12    23: 12    23: 56    24: 12
 25: 12    25: 56    26: 12    27: 12    28: 12    30: 12
 31: 12
 There are  23 hits at base# 12

-"-  gagtc                       1
```

26: 34

DdeI Ctnag 32

1: 14	2: 24	3: 14	3: 24	4: 14	4: 24
5: 24	6: 14	7: 14	7: 24	8: 14	9: 14
10: 14	11: 14	11: 24	12: 14	12: 24	15: 5
15: 14	16: 14	16: 24	19: 24	20: 14	23: 14
24: 14	25: 14	26: 14	27: 14	28: 14	29: 30
30: 14	31: 14				

There are 21 hits at base# 14

BsaJI Ccnngg 38

1: 23	1: 40	2: 39	2: 40	3: 39	3: 40
4: 39	4: 40	5: 39	11: 39	12: 38	12: 39
13: 23	13: 39	14: 23	14: 39	15: 38	16: 39
17: 23	17: 39	18: 23	18: 39	21: 38	21: 39
21: 47	22: 38	22: 39	22: 47	26: 40	27: 39
28: 39	29: 14	29: 39	30: 38	30: 39	30: 47
31: 23	31: 32				

There are 17 hits at base# 39

There are 5 hits at base# 38

There are 5 hits at base# 40 Makes cleavage ragged.

MnlI cctc 35

1: 23	2: 23	3: 23	4: 23	5: 23	6: 19
6: 23	7: 19	8: 23	9: 19	9: 23	10: 23
11: 23	13: 23	14: 23	16: 23	17: 23	18: 23
19: 23	20: 47	21: 23	21: 29	21: 47	22: 23
22: 29	22: 35	22: 47	23: 26	23: 29	24: 27
27: 23	28: 23	30: 35	30: 47	31: 23	

There are 21 hits at base# 23

There are 3 hits at base# 19

There are 3 hits at base# 29

There are 1 hits at base# 26

There are 1 hits at base# 27 These could make cleavage ragged.

-"- gagg 7

1: 48 2: 48 3: 48 4: 48 27: 44 28: 44
29: 44

BssKI Nccngg 39

1: 40 2: 39 3: 39 3: 40 4: 39 4: 40
5: 39 6: 31 6: 39 7: 31 7: 39 8: 39
9: 31 9: 39 10: 39 11: 39 12: 38 12: 52
13: 39 13: 52 14: 52 16: 39 16: 52 17: 39
17: 52 18: 39 18: 52 19: 39 19: 52 21: 38
22: 38 23: 39 24: 39 26: 39 27: 39 28: 39
29: 14 29: 39 30: 38
There are 21 hits at base# 39
There are 4 hits at base# 38
There are 3 hits at base# 31
There are 3 hits at base# 40 Ragged

BstNI CCwgg 30

1: 41 2: 40 5: 40 6: 40 7: 40 8: 40
9: 40 10: 40 11: 40 12: 39 12: 53 13: 40
13: 53 14: 53 16: 40 16: 53 17: 40 17: 53
18: 40 18: 53 19: 53 21: 39 22: 39 23: 40
24: 40 27: 40 28: 40 29: 15 29: 40 30: 39
There are 17 hits at base# 40
There are 7 hits at base# 53
There are 4 hits at base# 39
There are 1 hits at base# 41 Ragged

PspGI ccwgg 30

1: 41 2: 40 5: 40 6: 40 7: 40 8: 40
9: 40 10: 40 11: 40 12: 39 12: 53 13: 40
13: 53 14: 53 16: 40 16: 53 17: 40 17: 53
18: 40 18: 53 19: 53 21: 39 22: 39 23: 40
24: 40 27: 40 28: 40 29: 15 29: 40 30: 39
There are 17 hits at base# 40
There are 7 hits at base# 53

There are 4 hits at base# 39

There are 1 hits at base# 41

ScrFI CCngg 39

1: 41 2: 40 3: 40 3: 41 4: 40 4: 41
5: 40 6: 32 6: 40 7: 32 7: 40 8: 40
9: 32 9: 40 10: 40 11: 40 12: 39 12: 53
13: 40 13: 53 14: 53 16: 40 16: 53 17: 40
17: 53 18: 40 18: 53 19: 40 19: 53 21: 39
22: 39 23: 40 24: 40 26: 40 27: 40 28: 40
29: 15 29: 40 30: 39

There are 21 hits at base# 40

There are 4 hits at base# 39

There are 3 hits at base# 41

MaeIII gtnac 16

1: 52 2: 52 3: 52 4: 52 5: 52 6: 52
7: 52 9: 52 26: 52 27: 10 27: 52 28: 10
28: 52 29: 10 29: 52 30: 52

There are 13 hits at base# 52

Tsp45I gtsac 15

1: 52 2: 52 3: 52 4: 52 5: 52 6: 52
7: 52 9: 52 27: 10 27: 52 28: 10 28: 52
29: 10 29: 52 30: 52

There are 12 hits at base# 52

HphI tcacc 26

1: 53 2: 53 3: 53 4: 53 5: 53 6: 53
7: 53 8: 53 9: 53 10: 53 11: 59 13: 59
14: 59 17: 59 18: 59 19: 59 20: 59 21: 59
22: 59 23: 59 24: 59 25: 59 27: 59 28: 59
30: 59 31: 59

There are 16 hits at base# 59

There are 10 hits at base# 53

BspMI ACCTGCNNNNn 14

11: 61 13: 61 14: 61 17: 61 18: 61 19: 61

20: 61 21: 61 22: 61 23: 61 24: 61 25: 61

30: 61 31: 61

There are 14 hits at base# 61 Goes into CDR1

Table 12: Matches to URE FR3 adapters in 79 human HC.

A. List of Heavy-chains genes sampled

AF008566	AF103367	HSA235674	HSU94417	S83240
AF035043	AF103368	HSA235673	HSU94418	SABVH369
AF103026	AF103369	HSA240559	HSU96389	SADEIGVH
af103033	AF103370	HSCB201	HSU96391	SAH2IGVH
AF103061	af103371	HSIGGVHC	HSU96392	SDA3IGVH
Af103072	AF103372	HSU44791	HSU96395	SIGVHTTD
af103078	AF158381	HSU44793	HSZ93849	SUK4IGVH
AF103099	E05213	HSU82771	HSZ93850	
AF103102	E05886	HSU82949	HSZ93851	
AF103103	E05887	HSU82950	HSZ93853	
AF103174	HSA235661	HSU82952	HSZ93855	
AF103186	HSA235664	HSU82961	HSZ93857	
af103187	HSA235660	HSU86522	HSZ93860	
AF103195	HSA235659	HSU86523	HSZ93863	
af103277	HSA235678	HSU92452	MCOMFRAA	
af103286	HSA235677	HSU94412	MCOMFRVA	
AF103309	HSA235676	HSU94415	S82745	
af103343	HSA235675	HSU94416	S82764	

Table 12B. Testing all distinct GLGs from bases 89.1 to 93.2 of the heavy variable domain

Id	Nb	0	1	2	3	4		SEQ ID NO:
1	38	15	11	10	0	2	Seq1 gtgtattactgtgc	25
2	19	7	6	4	2	0	Seq2 gtAtattactgtgc	26
3	1	0	0	1	0	0	Seq3 gtgtattactgtAA	27
4	7	1	5	1	0	0	Seq4 gtgtattactgtAc	28
5	0	0	0	0	0	0	Seq5 Ttgtattactgtgc	29
6	0	0	0	0	0	0	Seq6 TtgtatCactgtgc	30
7	3	1	0	1	1	0	Seq7 ACAtattactgtgc	31
8	2	0	2	0	0	0	Seq8 ACgtattactgtgc	32
9	9	2	2	4	1	0	Seq9 ATgtattactgtgc	33
Group		26	26	21	4	2		
Cumulative		26	52	73	77	79		

Table 12C Most important URE recognition seqs in FR3 Heavy

1	VHSzy1	GTGtattactgtgc	(ON_SHC103)	(SEQ ID NO:25)
2	VHSzy2	GTAtattactgtgc	(ON_SHC323)	(SEQ ID NO:26)
3	VHSzy4	GTGtattactgtac	(ON_SHC349)	(SEQ ID NO:28)
4	VHSzy9	ATGtattactgtgc	(ON_SHC5a)	(SEQ ID NO:33)

Table 12D, testing 79 human HC V genes with four probes

Number of sequences.......... 79
Number of bases.............. 29143

		Number of mismatches						
Id	Best	0	1	2	3	4	5	
1	39	15	11	10	1	2	0	Seq1 gtgtattactgtgc (SEQ ID NO:25)
2	22	7	6	5	3	0	1	Seq2 gtAtattactgtgc (SEQ ID NO:26)
3	7	1	5	1	0	0	0	Seq4 gtgtattactgtAc (SEQ ID NO:28)
4	11	2	4	4	1	0	0	Seq9 ATgtattactgtgc (SEQ ID NO:33)
Group		25	26	20	5	2		
Cumulative		25	51	71	76	78		

One sequence has five mismatches with sequences 2, 4, and 9; it is scored as best for 2.

Id is the number of the adapter.
Best is the number of sequence for which the identified adapter was the best available.
The rest of the table shows how well the sequences match the adapters. For example, there are 10 sequences that match VHSzy1(Id=1) with 2 mismatches and are worse for all other adapters. In this sample, 90% come within 2 bases of one of the four adapters.

Table 13

The following list of enzymes was taken from
http://rebase.neb.com/cgi-bin/asymmlist.

I have removed the enzymes that a) cut within the recognition, b) cut on both sides of the recognition, or c) have fewer than 2 bases between recognition and closest cut site.

REBASE Enzymes
04/13/2001

Type II restriction enzymes with asymmetric recognition sequences:

Enzymes	Recognition Sequence	Isoschizomers	Suppliers	
AarI	CACCTGCNNNN^NNNN_	-	y	
AceIII	CAGCTCNNNNNNN^NNNN_	-	-	
Bbr7I	GAAGACNNNNNNN^NNNN_	-	-	
BbvI	GCAGCNNNNNNNN^NNNN_		y	
BbvII	GAAGACNN^NNNN_			
Bce83I	CTTGAGNNNNNNNNNNNNNN_NN^	-	-	
BceAI	ACGGCNNNNNNNNNNNN^NN_	-	y	
BcefI	ACGGCNNNNNNNNNNNN^N_	-	-	
BciVI	GTATCCNNNNN_N^	BfuI	y	
BfiI	ACTGGGNNNN_N^	BmrI	y	
BinI	GGATCNNNN^N_			
BscAI	GCATCNNNN^NN_	-	-	
BseRI	GAGGAGNNNNNNN_NN^	-	y	
BsmFI	GGGACNNNNNNNNN^NNNN_	BspLU11III	y	
BspMI	ACCTGCNNNN^NNNN_	Acc36I	y	
EciI	GGCGGANNNNNNNNN_NN^	-	y	
Eco57I	CTGAAGNNNNNNNNNNNNNN_NN^	BspKT5I	y	
FauI	CCCGCNNNN^NN_	BstFZ438I	y	
FokI	GGATGNNNNNNNN^NNNN_	BstPZ418I	y	
GsuI	CTGGAGNNNNNNNNNNNNNN_NN^	-	y	
HgaI	GACGCNNNNN^NNNNN_	-	y	
HphI	GGTGANNNNNNN_N^	AsuHPI	y	
MboII	GAAGANNNNNNN_N^	-	y	
MlyI	GAGTCNNNNN^	SchI	y	
MmeI	TCCRACNNNNNNNNNNNNNNNNNN_NN^		-	-
MnlI	CCTCNNNNNN_N^	-	y	
PleI	GAGTCNNNN^N_	PpsI	y	
RleAI	CCCACANNNNNNNNN_NNN^	-	-	
SfaNI	GCATCNNNNN^NNNN_	BspST5I	y	
SspD5I	GGTGANNNNNNNN^	-	-	
Sth132I	CCCGNNNN^NNNN_	-	-	
StsI	GGATGNNNNNNNNNN^NNNN_	-	-	
TaqII	GACCGANNNNNNNNN_NN^, CACCCANNNNNNNNN_NN^		-	-
Tth111II	CAARCANNNNNNNNN_NN^	-	-	
UbaPI	CGAACG	-	-	

The notation is ^ means cut the upper strand and _ means cut the lower strand. If the upper and lower strand are cut at the same place, then only ^ appears.

Table 14

(FOKIact) 5'-**cAcATccgTg** TTgTT **cAcggATgTg**-3'

(VHEx881) 5'-AATAgTAgAc TgcAgTgTcc TcAgcccTTA AgcTgTTcAT cTgcAAgTAg-
AgAgTATTcT TAgAgTTgTc TcTAgAcTTA gTgAAgcg-3'

! note that VHEx881 is the reverse complement of the ON below

! **[RC]** 5'-cgCttcacTaag-

! Scab.....

! Synthetic 3-23 as in Table 206

! |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-

! XbaI...

! |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3'

! AflII...

(VHBA881) 5'-cgCttcacTaag-
|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3'

(VHBB881) 5'-cgCttcacTaag-
|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'

(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'

Table 15: Use of *Fok*I as "Universal Restriction Enzyme"

*Fok*I - for dsDNA, | represents sites of cleavage

```
                              sites of cleavage
      5'-cacGGATGtg--nnnnnnn|nnnnnnn-3'(SEQ ID NO:15)
      3'-gtgCCTACac--nnnnnnnnnnn|nnn-5'(SEQ ID NO:16)
            RECOG
            NITion of FokI
```

Case I

```
            5'-...gtg|tatt-actgtgc..Substrate....-3' (SEQ ID NO:17)
               3'-cac-ataa|tgacacg¬
                                   gtGTAGGcac\
                                5'- caCATCCgtg/(SEQ ID NO:18)
```

Case II

```
            5'-...gtgtatt|agac-tgc..Substrate....-3'(SEQ ID NO:19)
                ┌cacataa-tctg|acg-5'
      /gtgCCTACac
      \cacGGATGtg-3'(SEQ ID NO:20)
```

Case III (Case I rotated 180 degrees)

```
      /gtgCCTACac-5'
      \cacGGATGtg¬
                  gtgtctt|acag-tcc-3' Adapter (SEQ ID NO:21)
            3'-...cacagaa-tgtc|agg..substrate....-5'(SEQ ID NO:22)
```

Case IV (Case II rotated 180 degrees)

```
                                3'- gtGTAGGcac\  (SEQ ID NO:23)
                                  ┌caCATCCgtg/
               5'-gag|tctc-actgagc
 Substrate 3'-...ctc-agag|tgactcg...-5'(SEQ ID NO:24)
```

Improved *Fok*I adapters

*Fok*I - for dsDNA, | represents sites of cleavage

Case I
Stem 11, loop 5, stem 11, recognition 17

```
            5'-...catgtg|tatt-actgtgc..Substrate....-3'
               3'-gtacac-ataa|tgacacg¬          ┌T¬
                                   gtGTAGGcacG  T
                                5'- caCATCCgtgc  C
                                              └TT┘
```

Case II
Stem 10, loop 5, stem 10, recognition 18

```
                5'-...gtgtatt|agac-tgctgcc..Substrate....-3'
          ┌T┐         ┌─cacataa-tctg|acgacgg-5'
 5        T  gtgCCTACac
          C  cacGGATGtg-3'
          └TT┘
```

Case III (Case I rotated 180 degrees)
Stem 11, loop 5, stem 11, recognition 20

```
10        ┌ T┐
          T  TgtgCCTACac-5'
          G  AcacGGATGtg─┐
          └TT┘           gtgtctt|acag-tccattctg-3' Adapter
                  3'-...cacagaa-tgtc|aggtaagac..substrate....-5'
```

15 Case IV (Case II rotated 180 degrees)
Stem 11, loop 4, stem 11, recognition 17

```
                                                ┌T┐
                                      3'- gtGTAGGcacc T
                                        ┌─caCATCCgtgg T
20                5'-atcgag|tctc-actgagc          └T┘
 Substrate 3'-...tagctc-agag|tgactcg...-5'
```

BseRI

```
                                  | sites of cleavage
      5'-cacGAGGAGnnnnnnnnnn|nnnnn-3'
25    3'-gtgctcctcnnnnnnnn|nnnnnnn-5'
            RECOG
            NITion of Bse RI
```

Stem 11, loop 5, stem 11, recognition 19

```
            3'-.......gaacat|cg-ttaagccagta.....5'
30      ┌T-T┐           cttgta-gc|aattcggtcat-3'
        C   GCTGAGGAGTC-┘
        T   cgactcctcag-5'  An adapter for BseRI to cleave the substrate above.
        └T──┘
```

Table 16 Human heavy chains bases 88.1 to 94.2

Number of sequences..... 840

Id	Ntot	Number of Mismatchers..... 0	1	2	3	4	5	6	7	Name	Probe Sequence.....	Dot form.....
1	364	152	97	76	26	7	4	2	0	VHS881-1.1	gctgtgtattactgtgcgag	gctgtgtattactgtgcgag
2	265	150	60	33	13	5	4	0	0	VHS881-1.2	gccgtgtattactgtgcgag	..c..............
3	96	14	34	16	10	5	7	9	1	VHS881-2.1	gccgtatattactgtgcgag	..c..a..........
4	20	0	3	4	9	2	2	0	0	VHS881-4.1	gccgtgtattactgtacgag	..c..........a....
5	95	25	36	18	11	2	2	0	1	VHS881-9.1	gccatgtattactgtgcgag	..ca..............
	840	341	230	147	69	21	19	11	2			
		341	571	718	787	808	827	838	840			

88 89 90 91 92 93 94 95 Codon number as in Table 195
Recognition..... Stem..... Loop. Stem.....

(VHS881-1.1) 5'-gctgtgtat|tact-gtgcgag **cAcATccgTg** TTgTT **cAcggATgTg**-3'
(VHS881-1.2) 5'-gccgtgtat|tact-gtgcgag **cAcATccgTg** TTgTT **cAcggATgTg**-3'
(VHS881-2.1) 5'-gccgtatat|tact-gtgcgag **cAcATccgTg** TTgTT **cAcggATgTg**-3'
(VHS881-4.1) 5'-gccgtgtat|tact-gtacgag **cAcATccgTg** TTgTT **cAcggATgTg**-3'
(VHS881-9.1) 5'-gccatgtat|tact-gtgcgag **cAcATccgTg** TTgTT **cAcggATgTg**-3'
| site of substrate cleavage

(FOKIact) 5'-**cAcATccgTg** TTgTT **cAcggATgTg**-3'

(VHEx881) 5'-AATAgTAgAc TgcAgTgTcc TcAgcccTTA AgcTgTTcAT cTgcAAgTAg-
AgAgTATTcT TAgAgTTgTc TcTAgAcTTA gTgAAgcg-3'
! note that VHEx881 is the reverse complement of the ON below
! **[RC]** 5'-cgCttcacTaag-
! Scab.....
! Synthetic 3-23 as in Table 206
! |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
! XbaI...

```
!              |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3'
!                  AflII...
(VHBA881)      5'-cgCttcacTaag-
               |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
               |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3'
(VHBB881)      5'-cgCttcacTaag-
               |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
               |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'
(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'
```

Table 17: Kappa, bases 12-30

ID	Ntot	0	1	2	3	4	5	6	Name	Sequence.....	Dot Form.....
1	84	40	21	20	1	2	0	0	SK12O12	gacccagtctccatcctcc	gacccagtctccatcctcc
2	32	19	3	6	2	1	0	1	SK12A17	gactcagtctccactctcc	...t.....ct....
3	26	17	8	1	0	0	0	0	SK12A27	gacgcagtctccaggcacc	...g.....gg.a..
4	40	21	18	1	0	0	0	0	SK12A11	gacgcagtctccagccacc	...g.....g..a..
	182	97	50	28	3	3	0	1			
		97	147	175	178	181	181	182			

URE adapters:

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-O12) 5'-cAcATccgTg TTgTT cAcggATgTg ggAggATggAgAcTgggTc-3'

[RC] 5'-gacccagtctccatcctcc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

FokI. FokI.

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-A17) 5'-cAcATccgTg TTgTT cAcggATgTg ggAgAgTggAgAcTgAgTc-3'

[RC] 5'-gactcagtctccactctcc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

FokI. FokI.

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-A27) 5'-cAcATccgTg TTgTT cAcggATgTg ggTgccTggAgAcTgcgTc-3'

[RC] 5'-gacgcagtctccaggcacc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

FokI. FokI.

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-A11) 5'-cAcATccgTg TTgTT cAcggATgTg ggTggcTggAgAcTgcgTc-3'

[RC] 5'-gacgcagtctccagccacc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

```
!                          FokI.        FokI.

What happens in the upper strand:

(SzKB1230-O12*)              5'-gac cca gtc|tcc a-tc ctc c-3'
!                             | Site of cleavage in substrate
!
(SzKB1230-A17*)              5'-gac tca gtc|tcc a-ct ctc c-3'
!
(SzKB1230-A27*)              5'-gac gca gtc|tcc a-gg cac c-3'
!
(SzKB1230-A11*)              5'-gac gca gtc|tcc a-gc cac c-3'

(kapextURE)      5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg-3' !sense strand
                    Scab.............ApaLI.

(kapextUREPCR)   5'-ccTctactctTgTcAcAgTg-3'
                    Scab............

(kaBRO1UR)     5'-ggAggATggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-tc ctc c-3' ON above is R.C. of this one
(kaBRO2UR)     5'-ggAgAgTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-ct ctc c-3' ON above is R.C. of this one
(kaBRO3UR)     5'-ggTgccTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gg cac c-3' ON above is R.C. of this one
(kaBRO4UR)     5'-ggTggcTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gc cac c-3' ON above is R.C. of this one
                 Scab.............ApaLI.
```

Table 18 Lambda URE adapters bases 13.3 to 19.3

! Number of sequences..... 128

Id	Ntot	Number of mismatches 0	1	2	3	4	5	6	7	8	Name	Sequence.....	Dot form.....
1	58	45	7	1	0	0	0	2	2	1	VL133-2a2	gtctcctggacagtcgatc	gtctcctggacagtcgatc
2	16	10	1	0	1	0	1	1	0	2	VL133-3l	ggccttgggacagacagtc	.g.cttg.....a.ag..
3	17	6	0	0	0	4	1	1	5	0	VL133-2c	gtctcctggacagtcagtc	.....ag..
4	37	3	0	10	4	4	3	7	4	2	VL133-1c	ggccccagggcagagggtc	.g.c..a..g...ag.g..
	128	64	8	11	5	8	5	11	11	5			
		64	72	83	88	96	101	112	123	128			

! Stem..... loop. Stem..... Recognition.....

(VL133-2a2) 5'-cAcATccgTg TTgTT cAcggATgTg gATcgAcTgTccAggAgAc-3'

[RC] 5'-gtctcctggacagtcgatc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

! Recognition..... Stem..... Loop. Stem.....

! Stem..... loop. Stem..... Recognition.....

(VL133-3l) 5'-cAcATccgTg TTgTT cAcggATgTg gAcTgTcTgTcccAAggcc-3'

[RC] 5'-ggccttgggacagacagtc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

! Recognition..... Stem..... Loop. Stem.....

! Stem..... loop. Stem..... Recognition.....

(VL133-2c) 5'-cAcATccgTg TTgTT cAcggATgTg gAcTgAcTgTccAggAgAc-3'

[RC] 5'-gtctcctggacagtcagtc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

! Recognition..... Stem..... Loop. Stem.....

! Stem..... loop. Stem..... Recognition.....

(VL133-1c) 5'-cAcATccgTg TTgTT cAcggATgTg gAcccTcTgcccTggggcc-3'

[RC] 5'-ggccccagggcagagggtc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

What happens in the top strand:

```
!                    | site of cleavage in the upper strand
(VL133-2a2*)   5'-g tct cct g|ga cag tcg atc
!
(VL133-3l*)    5'-g gcc ttg g|ga cag aca gtc
!
(VL133-2c*)    5'-g tct cct g|ga cag tca gtc
!
(VL133-1c*)    5'-g gcc cca g|gg cag agg gtc
!
! The following Extenders and Bridges all encode the AA sequence of 2a2 for codons 1-15
!                        1
(ON_LamEx133)  5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
       AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
       tcC ccG g ! 2a2
!                      1
(ON_LamB1-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
       AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
       tcC ccG g ga cag tcg at-3' ! 2a2  N.B. the actual seq is the
!                          reverse complement of the
!                          one shown.
!
(ON_LamB2-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
       AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
       tcC ccG g ga cag aca gt-3' ! 3l  N.B. the actual seq is the
!                          reverse complement of the
!                          one shown.
!
!
(ON_LamB3-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
       AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
       tcC ccG g ga cag tca gt -3'! 2c  N.B. the actual seq is the
!                          reverse complement of the
!                          one shown.
!(ON_LamB4-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
```

!
! 2 3 4 5 6 7 8 9 10 11 12
AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-s
!
! 13 14 15
tcC ccG g gg cag agg gt-3' ! 1c **N.B.** the actual seq is the reverse complement of the one shown.
!
!
!
(ON_Lam133PCR) 5'-ccTcTgAcTgA**gT gcA c**Ag AGt gc-3'

Table 19: Cleavage of 75 human light chains.

Enzyme	Recognition*	Nch	Ns	Planned location of site
AfeI	AGCgct	0	0	
AflII	**Cttaag**	**0**	**0**	**HC FR3**
AgeI	Accggt	0	0	
AscI	**GGcgcgcc**	**0**	**0**	**After LC**
BglII	Agatct	0	0	
BsiWI	Cgtacg	0	0	
BspDI	ATcgat	0	0	
BssHII	Gcgcgc	0	0	
BstBI	TTcgaa	0	0	
DraIII	CACNNNgtg	0	0	
EagI	Cggccg	0	0	
FseI	GGCCGGcc	0	0	
FspI	TGCgca	0	0	
HpaI	GTTaac	0	0	
MfeI	**Caattg**	**0**	**0**	**HC FR1**
MluI	Acgcgt	0	0	
NcoI	**Ccatgg**	**0**	**0**	**Heavy chain signal**
NheI	**Gctagc**	**0**	**0**	**HC/anchor linker**
NotI	**GCggccgc**	**0**	**0**	**In linker after HC**
NruI	TCGcga	0	0	
PacI	TTAATtaa	0	0	
PmeI	GTTTaaac	0	0	
PmlI	CACgtg	0	0	
PvuI	CGATcg	0	0	
SacII	CCGCgg	0	0	
SalI	Gtcgac	0	0	
SfiI	**GGCCNNNNnggcc**	**0**	**0**	**Heavy Chain signal**
SgfI	GCGATcgc	0	0	
SnaBI	TACgta	0	0	
StuI	AGGcct	0	0	
XbaI	**Tctaga**	**0**	**0**	**HC FR3**
AatII	GACGTc	1	1	
AclI	AAcgtt	1	1	
AseI	ATtaat	1	1	
BsmI	GAATGCN	1	1	
BspEI	**Tccgga**	**1**	**1**	**HC FR1**
BstXI	**CCANNNNNntgg**	**1**	**1**	**HC FR2**
DrdI	GACNNNNnngtc	1	1	
HindIII	Aagctt	1	1	
PciI	Acatgt	1	1	
SapI	gaagagc	1	1	
ScaI	AGTact	1	1	
SexAI	Accwggt	1	1	
SpeI	Actagt	1	1	
TliI	Ctcgag	1	1	
XhoI	Ctcgag	1	1	
BcgI	cgannnnnntgc	2	2	
BlpI	GCtnagc	2	2	
BssSI	Ctcgtg	2	2	
BstAPI	GCANNNNntgc	2	2	
EspI	GCtnagc	2	2	
KasI	Ggcgcc	2	2	
PflMI	CCANNNNntgg	2	2	
XmnI	GAANNnnttc	2	2	
ApaLI	**Gtgcac**	**3**	**3**	**LC signal seq**

NaeI	GCCggc	3	3	
NgoMI	Gccggc	3	3	
PvuII	CAGctg	3	3	
RsrII	CGgwccg	3	3	
BsrBI	GAGcgg	4	4	
BsrDI	GCAATGNNn	4	4	
BstZ17I	GTAtac	4	4	
EcoRI	Gaattc	4	4	
SphI	GCATGc	4	4	
SspI	AATatt	4	4	
AccI	GTmkac	5	5	
BclI	Tgatca	5	5	
BsmBI	Nnnnnngagacg	5	5	
BsrGI	Tgtaca	5	5	
DraI	TTTaaa	6	6	
NdeI	**CAtatg**	**6**	**6**	**HC FR4**
SwaI	ATTTaaat	6	6	
BamHI	Ggatcc	7	7	
SacI	GAGCTc	7	7	
BciVI	GTATCCNNNNNN	8	8	
BsaBI	GATNNnnatc	8	8	
NsiI	ATGCAt	8	8	
Bsp120I	**Gggccc**	**9**	**9**	**CH1**
ApaI	**GGGCCc**	**9**	**9**	**CH1**
PspOOMI	Gggccc	9	9	
BspHI	Tcatga	9	11	
EcoRV	GATatc	9	9	
AhdI	GACNNNnngtc	11	11	
BbsI	GAAGAC	11	14	
PsiI	TTAtaa	12	12	
BsaI	GGTCTCNnnnn	13	15	
XmaI	Cccggg	13	14	
AvaI	Cycgrg	14	16	
BglI	GCCNNNNnggc	14	17	
AlwNI	CAGNNNctg	16	16	
BspMI	ACCTGC	17	19	
XcmI	CCANNNNNnnnntgg	17	26	
BstEII	**Ggtnacc**	**19**	**22**	**HC FR4**
Sse8387I	CCTGCAgg	20	20	
AvrII	Cctagg	22	22	
HincII	GTYrac	22	22	
BsgI	GTGCAG	27	29	
MscI	TGGcca	30	34	
BseRI	NNnnnnnnnnctcctc	32	35	
Bsu36I	CCtnagg	35	37	
PstI	CTGCAg	35	40	
EciI	nnnnnnnnntccgcc	38	40	
PpuMI	RGgwccy	41	50	
StyI	Ccwwgg	44	73	
EcoO109I	RGgnccy	46	70	
Acc65I	Ggtacc	50	51	
KpnI	GGTACc	50	51	
BpmI	ctccag	53	82	
AvaII	Ggwcc	71	124	

* cleavage occurs in the top strand after the last upper-case base. For REs that cut palindromic sequences, the lower strand is cut at the symmetrical site.

Table 20: Cleavage of 79 human heavy chains

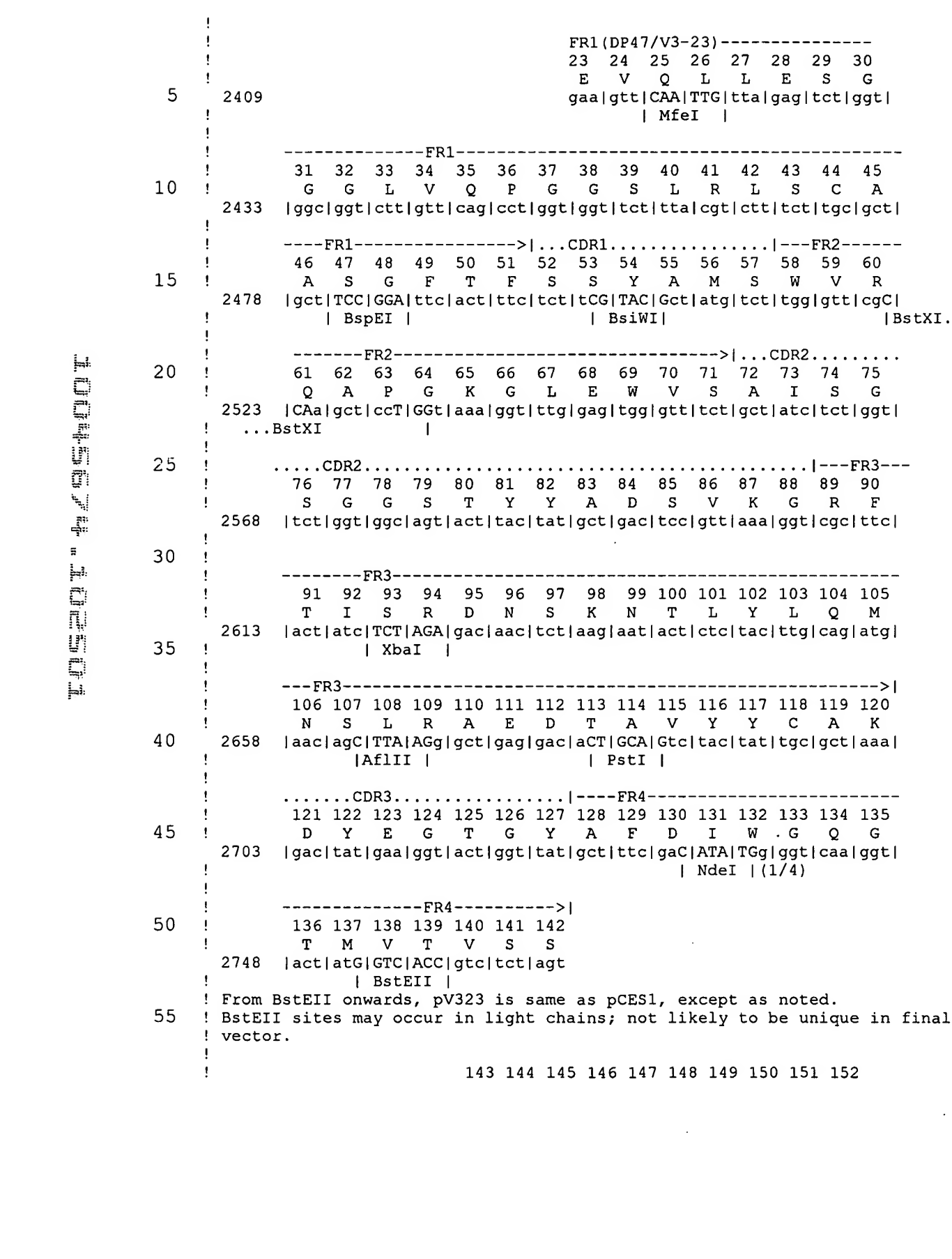
Enzyme	Recognition	Nch	Ns	Planned location of site
AfeI	AGCgct	0	0	
AflII	**Cttaag**	**0**	**0**	**HC FR3**
AscI	**GGcgcgcc**	**0**	**0**	**After LC**
BsiWI	Cgtacg	0	0	
BspDI	ATcgat	0	0	
BssHII	Gcgcgc	0	0	
FseI	GGCCGGcc	0	0	
HpaI	GTTaac	0	0	
NheI	**Gctagc**	**0**	**0**	**HC Linker**
NotI	**GCggccgc**	**0**	**0**	**In linker, HC/anchor**
NruI	TCGcga	0	0	
NsiI	ATGCAt	0	0	
PacI	TTAATtaa	0	0	
PciI	Acatgt	0	0	
PmeI	GTTTaaac	0	0	
PvuI	CGATcg	0	0	
RsrII	CGgwccg	0	0	
SapI	gaagagc	0	0	
SfiI	**GGCCNNNNnggcc**	**0**	**0**	**HC signal seq**
SgfI	GCGATcgc	0	0	
SwaI	ATTTaaat	0	0	
AclI	AAcgtt	1	1	
AgeI	Accggt	1	1	
AseI	ATtaat	1	1	
AvrII	Cctagg	1	1	
BsmI	GAATGCN	1	1	
BsrBI	GAGcgg	1	1	
BsrDI	GCAATGNNn	1	1	
DraI	TTTaaa	1	1	
FspI	TGCgca	1	1	
HindIII	Aagctt	1	1	
MfeI	**Caattg**	**1**	**1**	**HC FR1**
NaeI	GCCggc	1	1	
NgoMI	Gccggc	1	1	
SpeI	Actagt	1	1	
Acc65I	Ggtacc	2	2	
BstBI	TTcgaa	2	2	
KpnI	GGTACc	2	2	
MluI	Acgcgt	2	2	
NcoI	**Ccatgg**	**2**	**2**	**In HC signal seq**
NdeI	**CAtatg**	**2**	**2**	**HC FR4**
PmlI	CACgtg	2	2	
XcmI	CCANNNNNnnnntgg	2	2	
BcgI	cgannnnnntgc	3	3	
BclI	Tgatca	3	3	
BglI	GCCNNNNnggc	3	3	
BsaBI	GATNNnnatc	3	3	
BsrGI	Tgtaca	3	3	
SnaBI	TACgta	3	3	
Sse8387I	CCTGCAgg	3	3	
ApaLI	**Gtgcac**	**4**	**4**	**LC Signal/FR1**
BspHI	Tcatga	4	4	
BssSI	Ctcgtg	4	4	
PsiI	TTAtaa	4	5	

SphI	GCATGc	4	4	
AhdI	GACNNNnngtc	5	5	
BspEI	**Tccgga**	**5**	**5**	**HC FR1**
MscI	TGGcca	5	5	
SacI	GAGCTc	5	5	
ScaI	AGTact	5	5	
SexAI	Accwggt	5	6	
SspI	AATatt	5	5	
TliI	Ctcgag	5	5	
XhoI	Ctcgag	5	5	
BbsI	GAAGAC	7	8	
BstAPI	GCANNNNntgc	7	8	
BstZ17I	GTAtac	7	7	
EcoRV	GATatc	7	7	
EcoRI	Gaattc	8	8	
BlpI	GCtnagc	9	9	
Bsu36I	CCtnagg	9	9	
DraIII	CACNNNgtg	9	9	
EspI	GCtnagc	9	9	
StuI	AGGcct	9	13	
XbaI	**Tctaga**	**9**	**9**	**HC FR3**
Bsp120I	**Gggccc**	**10**	**11**	**CH1**
ApaI	**GGGCCc**	**10**	**11**	**CH1**
PspOOMI	Gggccc	10	11	
BciVI	GTATCCNNNNNN	11	11	
SalI	Gtcgac	11	12	
DrdI	GACNNNNnngtc	12	12	
KasI	Ggcgcc	12	12	
XmaI	Cccggg	12	14	
BglII	Agatct	14	14	
HincII	GTYrac	16	18	
BamHI	Ggatcc	17	17	
PflMI	CCANNNNntgg	17	18	
BsmBI	Nnnnnngagacg	18	21	
BstXI	**CCANNNNNntgg**	**18**	**19**	**HC FR2**
XmnI	GAANNnnttc	18	18	
SacII	CCGCgg	19	19	
PstI	CTGCAg	20	24	
PvuII	CAGctg	20	22	
AvaI	Cycgrg	21	24	
EagI	Cggccg	21	22	
AatII	GACGTc	22	22	
BspMI	ACCTGC	27	33	
AccI	GTmkac	30	43	
StyI	Ccwwgg	36	49	
AlwNI	CAGNNNctg	38	44	
BsaI	GGTCTCNnnnn	38	44	
PpuMI	RGgwccy	43	46	
BsgI	GTGCAG	44	54	
BseRI	NNnnnnnnnnctcctc	48	60	
EciI	nnnnnnnnntccgcc	52	57	
BstEII	**Ggtnacc**	**54**	**61**	**HC Fr4, 47/79 have one**
Eco0109I	RGgnccy	54	86	
BpmI	ctccag	60	121	
AvaII	Ggwcc	71	140	

Table 21: MALIA3, annotated

```
! MALIA3  9532 bases
!-------------------------------------------------------------------------
     1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc gcc
!    gene ii continued
    49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat gta
    97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca act
   145 gtt aca tgg aat gaa act tcc aga cac cgt act tta gtt gca tat tta
   193 aaa cat gtt gag cta cag cac cag att cag caa tta agc tct aag cca
   241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc tct
   289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa gct
   337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat ctt
   385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa gac
   433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa gca
   481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg gac
!          RBS?......      Start gene x, ii continues
   529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act tct
   577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta aac
   625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt tgg
   673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa ctg
   721 atg aat ctt tct acc tgt aat aat gtt gtt ccg tta gtt cgt ttt att
   769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca gtt
   817 ctt aaa atc gca TAA
!                    End X & II
   832 ggtaattca ca
!
!       M1              E5                 Q10                 T15
   843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act cgt
!      Start gene V
!
!      S17         S20                 P25                 E30
   891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag ctt
!
!              V35                 E40                 V45
   939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att act
!
!          D50                 A55                 L60
   987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt cat
!                                                   BsrGI...
!      L65                 V70                 S75                 R80
  1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac cgt
!
!                     P85     K87 end of V
  1083 ctg cgc ctc gtt ccg gct aag TAA C
!
  1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg
!      Start gene VII
!
  1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc
!
!                       VII and IX overlap.
!                       ..... S2  V3  L4  V5                 S10
  1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttc gcc tct ttc gtt
!                         End VII
!                        |start IX
!      L13     W15                 G20                 T25             E29
  1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta atg gaa
```

```
!
  1293 act tcc tc
!
!       .... stop of IX, IX and VIII overlap by four bases
  1301 ATG aaa aag tct tta gtc ctc aaa gcc tct gta gcc gtt gct acc ctc
!      Start signal sequence of viii.
!
  1349 gtt ccg atg ctg tct ttc gct gct gag ggt gac gat ccc gca aaa gcg
!                                  mature VIII --->
  1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat gcg
  1445 tgg gcg atg gtt gtt gtc att
  1466 gtc ggc gca act atc ggt atc aag ctg ttt aag
  1499 aaa ttc acc tcg aaa gca ! 1515
!       ..........  -35  ..
!
  1517       agc tga taaaccgat acaattaaag gctccttttg
!                     ..... -10   ...
!
  1552 gagccttttt ttttGGAGAt ttt ! S.D. underlined
!
!           <------ III signal sequence ----------------------------->
!            M   K   K   L   L   F   A   I   P   L   V
  1575 caac GTG aaa aaa tta tta ttc gca att cct tta gtt ! 1611
!
!       V   P   F   Y   S   H   S   A   Q
  1612 gtt cct ttc tat tct cac aGT gcA Cag tCT
!                            ApaLI...
!
  1642      GTC GTG ACG CAG CCG CCC TCA GTG TCT GGG GCC CCA GGG CAG
            AGG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC ATC GGG GCA
!             BstEII...
  1729      GGT TAT GAT GTA CAC TGG TAC CAG CAG CTT CCA GGA ACA GCC CCC AAA
  1777      CTC CTC ATC TAT GGT AAC AGC AAT CGG CCC TCA GGG GTC CCT GAC CGA
  1825      TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC ACT
  1870      GGG CTC CAG GCT GAG GAT GAG GCT GAT TAT
  1900      TAC TGC CAG TCC TAT GAC AGC AGC CTG AGT
  1930      GGC CTT TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC
!                                              BstEII...
  1969      CTA GGT CAG CCC AAG GCC AAC CCC ACT GTC ACT
  2002      CTG TTC CCG CCC TCC TCT GAG GAG CTC CAA GCC AAC AAG GCC ACA CTA
  2050      GTG TGT CTG ATC AGT GAC TTC TAC CCG GGA GCT GTG ACA GTG GCC TGG
  2098      AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC
  2146      TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAT CTG AGC CTG
  2194      ACG CCT GAG CAG TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG
  2242      CAT GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC CCT ACA GAA TGT TCA
  2290      TAA TAA ACCG CCTCCACCGG GCGCGCCAAT TCTATTTCAA GGAGACAGTC ATA
!                              AscI.....
!
!           PelB signal---------------------------------------------->
!            M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
  2343      ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
!
!           16  17  18  19  20      21  22
!           A   A   Q   P   A       M   A
  2388     gcG GCC cag ccG GCC       atg gcc
!            SfiI.............
!                    NgoMI...(1/2)
!                           NcoI.........
```


FR1(DP47/V3-23)----------
23 24 25 26 27 28 29 30
E V Q L L E S G
2409
gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
| MfeI |
----------FR1----------
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
G G L V Q P G G S L R L S C A
2433 |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
----FR1----------->|...CDR1.....|---FR2------
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
A S G F T F S S Y A M S W V R
2478 |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|
| BspEI | | BsiWI| |BstXI.
-------FR2---------->|...CDR2.....
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Q A P G K G L E W V S A I S G
2523 |CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|
...BstXI |
.....CDR2.....|---FR3---
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
S G G S T Y Y A D S V K G R F
2568 |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|
--------FR3----------
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T I S R D N S K N T L Y L Q M
2613 |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
| XbaI |
---FR3----------->|
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
N S L R A E D T A V Y Y C A K
2658 |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|
|AflII | | PstI |
.......CDR3.....|----FR4----------
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
D Y E G T G Y A F D I W G Q G
2703 |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|
| NdeI |(1/4)
----------FR4---------->|
136 137 138 139 140 141 142
T M V T V S S
2748 |act|atG|GTC|ACC|gtc|tct|agt
| BstEII |
From BstEII onwards, pV323 is same as pCES1, except as noted.
BstEII sites may occur in light chains; not likely to be unique in final vector.
143 144 145 146 147 148 149 150 151 152

```
                                A   S   T   K   G   P   S   V   F   P
2769                            gcc tcc acc aaG GGC CCa tcg GTC TTC ccc
                                              Bsp120I.      BbsI...(2/2)
                                              ApaI....

     153 154 155 156 157 158 159 160 161 162 163 164 165 166 167
      L   A   P   S   S   K   S   T   S   G   G   T   A   A   L
2799 ctg gca ccC TCC TCc aag agc acc tct ggg ggc aca gcg gcc ctg
           BseRI...(2/2)

       168 169 170 171 172 173 174 175 176 177 178 179 180 181 182
        G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S
2844   ggc tgc ctg GTC AAG GAC TAC TTC CCc gaA CCG GTg acg gtg tcg
                                          AgeI....

       183 184 185 186 187 188 189 190 191 192 193 194 195 196 197
        W   N   S   G   A   L   T   S   G   V   H   T   F   P   A
2889   tgg aac tca GGC GCC ctg acc agc ggc gtc cac acc ttc ccg gct
           KasI...(1/4)

       198 199 200 201 202 203 204 205 206 207 208 209 210 211 212
        V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T
2934   gtc cta cag tCt agc GGa ctc tac tcc ctc agc agc gta gtg acc
           (Bsu36I...)(knocked out)

       213 214 215 216 217 218 219 220 221 222 223 224 225 226 227
        V   P   S   S   S   L   G   T   Q   T   Y   I   C   N   V
2979   gtg ccC tCt tct agc tTG Ggc acc cag acc tac atc tgc aac gtg
           (BstXI...........)N.B. destruction of BstXI & BpmI sites.

       228 229 230 231 232 233 234 235 236 237 238 239 240 241 242
        N   H   K   P   S   N   T   K   V   D   K   K   V   E   P
3024   aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc

       243 244 245
        K   S   C   A   A   A   H   H   H   H   H   H   S   A
3069   aaa tct tgt GCG GCC GCt cat cac cac cat cat cac tct gct
           NotI......

        E   Q   K   L   I   S   E   E   D   L   N   G   A   A
3111   gaa caa aaa ctc atc tca gaa gag gat ctg aat ggt gcc gca


        D   I   N   D   D   R   M     A   S   G   A
3153   GAT ATC aac gat gat cgt atg   gct AGC  ggc gcc
       rEK cleavage site.........   NheI...  KasI...
       EcoRV..

Domain 1 ------------------------------------------------------------
          A   E   T   V   E   S   C   L   A
3183     gct gaa act gtt gaa agt tgt tta gca


      K   P   H   T   E   I   S   F
3210 aaa ccc cat aca gaa aat tca ttt

      T   N   V   W   K   D   D   K   T
3234 aCT AAC GTC TGG AAA GAC GAC AAA ACt
```

```
!
!       L   D   R   Y   A   N   Y   E   G   C   L   W   N   A   T   G   V
  3261 tta gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc gtt
!                                                       BsmI____
!
!       V   V   C   T   G   D   E   T   Q   C   Y   G   T   W   V   P   I
  3312 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT cct att
!
!       G   L   A   I   P   E   N
  3363 ggg ctt gct atc cct gaa aat
!
! L1 linker ------------------------------------
!       E   G   G   G   S   E   G   G   G   S
  3384 gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!       E   G   G   G   S   E   G   G   G   T
  3414 gag ggt ggc ggt tct gag ggt ggc ggt act
!
!  Domain 2 -------------------------------------
  3444 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac
  3495 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct
  3546 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag aat
!                     BseRI__
  3597 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc act
  3645 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act cct
  3693 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC AGA
!                                                          AlwNI
  3741 GAC TGc gct ttc cat tct ggc ttt aat gaa gat cca ttc gtt tgt gaa
!       AlwNI
  3789 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct
!
  3834 ggc ggc ggc tct
! start L2 ------------------------------------------------------------
  3846 ggt ggt ggt tct
  3858 ggt ggc ggc tct
  3870 gag ggt ggt ggc tct gag ggt ggc ggt tct
  3900 gag ggt ggc ggc tct gag gga ggc ggt tcc
  3930 ggt ggt ggc tct ggt    ! end L2
!
! Domain 3 ------------------------------------------------------------
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  3945 tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct
!
!       M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
  3993 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc
!
!       K   L   D   S   V   A   T   D   Y   G   A   A   I   D   G   F
  4041 aaa ctt gat tct gtc gct act gat tac ggt gct gct atc gat ggt ttc
!
!       I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  4089 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat
!
!       F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  4137 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat
!
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  4185 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa
!
```

```
!       S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
  4233 tcg gtt gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa
!
!       F   S   I   D   C   D   K   I   N   L   F   R
  4281 ttt tct att gat tgt gac aaa ata aac tta ttc cgt
!                                                   End Domain 3
!
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V  F140
  4317 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt
!      start transmembrane segment
!
!       S   T   F   A   N   I   L
  4365 tct acg ttt gct aac ata ctg
!
!       R   N   K   E   S
  4386 cgt aat aag gag tct TAA ! stop of iii
!     Intracellular anchor.
!
!           M1  P2  V   L  L5   G   I   P   L  L10  L   R   F   L  G15
  4404  tc ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt
!          Start VI
!
  4451 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa aag
  4499 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt att
  4547 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc gct
  4595 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg tct
  4643 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct att
  4691 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg gat
!
!                 M1  A2  V3      F5                  L10         G13
  4739 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga
!       end VI   Start gene I
!
!       14  15  16  17  18  19  20  21  22  23  24  25  26  27  28
!       K   T   L   V   S   V   G   K   I   Q   D   K   I   V   A
  4785 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct
!
!       29  30  31  32  33  34  35  36  37  38  39  40  41  42  43
!       G   C   K   I   A   T   N   L   D   L   R   L   Q   N   L
  4830 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc
!
!       44  45  46  47  48  49  50  51  52  53  54  55  56  57  58
!       P   Q   V   G   R   F   A   K   T   P   R   V   L   R   I
  4875 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata
!
!       59  60  61  62  63  64  65  66  67  68  69  70  71  72  73
!       P   D   K   P   S   I   S   D   L   L   A   I   G   R   G
  4920 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt
!
!       74  75  76  77  78  79  80  81  82  83  84  85  86  87  88
!       N   D   S   Y   D   E   N   K   N   G   L   L   V   L   D
  4965 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat
!
!       89  90  91  92  93  94  95  96  97  98  99 100 101 102 103
!       E   C   G   T   W   F   N   T   R   S   W   N   D   K   E
  5010 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa
!
!      104 105 106 107 108 109 110 111 112 113 114 115 116 117 118
```

```
!       R   Q   P   I   I   D   W   F   L   H   A   R   K   L   G
  5055 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta gga
!
!      119 120 121 122 123 124 125 126 127 128 129 130 131 132 133
!       W   D   I   I   F   L   V   Q   D   L   S   I   V   D   K
  5100 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa
!
!      134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
!       Q   A   R   S   A   L   A   E   H   V   V   Y   C   R   R
  5145 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt
!
!      149 150 151 152 153 154 155 156 157 158 159 160 161 162 163
!       L   D   R   I   T   L   P   F   V   G   T   L   Y   S   L
  5190 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt
!
!      164 165 166 167 168 169 170 171 172 173 174 175 176 177 178
!       I   T   G   S   K   M   P   L   P   K   L   H   V   G   V
  5235 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt
!
!      179 180 181 182 183 184 185 186 187 188 189 190 191 192 193
!       V   K   Y   G   D   S   Q   L   S   P   T   V   E   R   W
  5280 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg
!
!      194 195 196 197 198 199 200 201 202 203 204 205 206 207 208
!       L   Y   T   G   K   N   L   Y   N   A   Y   D   T   K   Q
  5325 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag
!
!      209 210 211 212 213 214 215 216 217 218 219 220 221 222 223
!       A   F   S   S   N   Y   D   S   G   V   Y   S   Y   L   T
  5370 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg
!
!      224 225 226 227 228 229 230 231 232 233 234 235 236 237 238
!       P   Y   L   S   H   G   R   Y   F   K   P   L   N   L   G
  5415 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt
!
!      239 240 241 242 243 244 245 246 247 248 249 250 251 252 253
!       Q   K   M   K   L   T   K   I   Y   L   K   K   F   S   R
  5460 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc
!
!      254 255 256 257 258 259 260 261 262 263 264 265 266 267 268
!       V   L   C   L   A   I   G   F   A   S   A   F   T   Y   S
  5505 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt
!
!      269 270 271 272 273 274 275 276 277 278 279 280 281 282 283
!       Y   I   T   Q   P   K   P   E   V   K   K   V   V   S   Q
  5550 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag
!
!      284 285 286 287 288 289 290 291 292 293 294 295 296 297 298
!       T   Y   D   F   D   K   F   T   I   D   S   S   Q   R   L
  5595 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt
!
!      299 300 301 302 303 304 305 306 307 308 309 310 311 312 313
!       N   L   S   Y   R   Y   V   F   K   D   S   K   G   K   L
  5640 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA
!                                                               PacI
!
!      314 315 316 317 318 319 320 321 322 323 324 325 326 327 328
!       I   N   S   D   D   L   Q   K   Q   G   Y   S   L   T   Y
```

```
  5685 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat
!     PacI
!
!      329 330 331 332 333 334 335 336 337 338 339 340 341 342 343
5     !      i  I   D   L   C   T   V   S   I   K   K   G   N   S   N   E
!     iv                                                              M1  K
  5730   att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa
!                                                                    Start IV
!
10    !         344 345 346 347 348 349
!     i    I   V   K   C   N   .End of I
!     iv    L3  L   N5  V   I7  N    F  V10
  5775    att gtt aaa tgt aat TAA T TTT GTT
!  IV continued.....
15    5800 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa atg
      5848 aat aat tcg cct ctg cgc gat ttt gta act tgg tat tca aag caa tca
      5896 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act gta
      5944 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att tct
      5992 gtt tta cgt gct aat aat ttt gat atg gtt ggt tca att cct tcc ata
20    6040 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg cca
      6088 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt ggt
      6136 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att aat
      6184 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta aag
      6232 tct aat act tct aaa tcc tca aat gta tta tct att gac ggc tct aat
25    6280 cta tta gtt gtt TCT gca cct aaa gat att tta gat aac ctt cct caa
!                        ApaLI removed
      6328 ttc ctt tct act gtt gat ttg cca act gac cag ata ttg att gag ggt
      6376 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt gct
      6424 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac cgc
30    6472 ctc acc tct gtt tta tct tct gct ggt ggt tcg ttc ggt att ttt aat
      6520 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc cat
      6568 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag aag
      6616 ggt tct atc tct gtT GGC CAg aat gtc cct ttt att act ggt cgt gtg
!                      MscI____
35    6664 act ggt gaa tct gcc aat gta aat aat cca ttt cag acg att gag cgt
      6712 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct ggc
      6760 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt tct
      6808 tct act cag gca agt gat gtt att act aat caa aga agt att gct aca
      6856 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc act
40    6904 gat tat aaa aac act tct caa gat tct ggc gta ccg ttc ctg tct aaa
      6952 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcc aac gag
      7000 gaa agc acg tta tac gtg ctc gtc aaa gca acc ata gta cgc gcc ctg
      7048 TAG cggcgcatt
!     End IV
45    7060 aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccctagc
      7120 gcccgctcct ttcgctttct tcccttcctt tctcgccacg ttcGCCGGCt ttccccgtca
!                                                      NgoMI_
      7180 agctctaaat cgggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc
      7240 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tcgccctgat agacggtttt
50    !                                DraIII____
      7300 tcgcccttTG ACGTTGGAGT Ccacgttctt taatagtgga ctcttgttcc aaactggaac
!             DrdI__________
      7360 aacactcaac cctatctcgg gctattcttt tgatttataa gggattttgc cgatttcgga
      7420 accaccatca aacaggattt tcgcctgctg gggcaaacca gcgtggaccg cttgctgcaa
55    7480 ctctctcagg gccaggcggt gaagggcaat CAGCTGttgc cCGTCTCact ggtgaaaaga
!                                      PvuII.      BsmBI.
      7540 aaaaccaccc tGGATCC  AAGCTT
!                BamHI   HindIII (1/2)
```

```
!                      Insert carrying bla gene
 7563     gcaggtg gcacttttcg gggaaatgtg cgcggaaccc
 7600 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga caataaccct
!                                                 BciVI
 7660 gataaatgct tcaataatat tgaaaaAGGA AGAgt
!                                 RBS.?...
!     Start bla gene
 7695 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt
 7746 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct
 7797 gaa gat cag ttg ggC gCA CGA Gtg ggt tac atc gaa ctg gat ctc aac agc
!                             BssSI...
!                        ApaLI removed
 7848 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc
 7899 act ttt aaa gtt ctg cta tgt cat aca cta tta tcc cgt att gac gcc ggg
 7950 caa gaG CAA CTC GGT CGc cgg gcg cgg tat tct cag aat gac ttg gtt gAG
!         BcgI_____________                                         ScaI
 8001 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa
!     ScaI_
 8052 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt
 8103 ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg cac aac atg
!             PvuI____
 8154 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc
 8205 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg gca aca acg
 8256 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa caa
!      FspI....
!
 8307 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg cgc tcg
 8358 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt
!     BglI___________
 8409 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt
!      BsaI____
 8460 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa cga aat
!                          AhdI___________
 8511 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA ctgt
!                                                          stop
 8560 cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa
 8620 ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt
 8680 cgttccactg tacgtaagac cccc
 8704 AAGCTT   GTCGAC tgaa tggcgaatgg cgctttgcct
!     HindIII  SalI..
!     (2/2)    HincII
 8740 ggtttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt
!
 8790 CCTGAGG
!     Bsu36I_
 8797       ccgat actgtcgtcg tcccctcaaa ctggcagatg
 8832 cacggttacg atgcgcccat ctacaccaac gtaacctatc ccattacggt caatccgccg
 8892 tttgttccca cggagaatcc gacgggttgt tactcgctca catttaatgt tgatgaaagc
 8952 tggctacagg aaggccagac gcgaattatt tttgatggcg ttcctattgg ttaaaaaatg
 9012 agctgattta acaaaaattt aacgcgaatt ttaacaaaat attaacgttt acaATTTAAA
!                                                            SwaI...
 9072 Tatttgctta tacaatcttc ctgtttttgg ggcttttctg attatcaacc GGGGTAcat
!                                                         RBS?
 9131 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt gtt tgc
!     Start gene II
 9182 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca aaa ata
!                                                BglII...
 9233 gct acc ctc tcc ggc atg aat tta tca gct aga acg gtt gaa tat cat att
```

9284 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct tta cct
9335 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa aat ttt
9386 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag ggt cat
9437 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta ttg ctt
9488 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt ! 9532
! gene II continues

Table 21B: Sequence of MALIA3, condensed

LOCUS MALIA3 9532 CIRCULAR

ORIGIN

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        1 AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC AAATGAAAAT
       61 ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA ATGGTCAAAC TAAATCTACT
      121 CGTTCGCAGA ATTGGGAATC AACTGTTACA TGGAATGAAA CTTCCAGACA CCGTACTTTA
      181 GTTGCATATT TAAAACATGT TGAGCTACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA
      241 TCCGCAAAAA TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
      361 TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT TTGCTTCTGA CTATAATAGT
      421 CAGGGTAAAG ACCTGATTTT TGATTTATGG TCATTCTCGT TTTCTGAACT GTTTAAAGCA
      481 TTTGAGGGGG ATTCAATGAA TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT
      541 AAACATTTTA CTATTACCCC CTCTGGCAAA ACTTCTTTTG CAAAAGCCTC TCGCTATTTT
      601 GGTTTTTATC GTCGTCTGGT AAACGAGGGT TATGATAGTG TTGCTCTTAC TATGCCTCGT
      661 AATTCCTTTT GGCGTTATGT ATCTGCATTA GTTGAATGTG GTATTCCTAA ATCTCAACTG
      721 ATGAATCTTT CTACCTGTAA TAATGTTGTT CCGTTAGTTC GTTTTATTAA CGTAGATTTT
      781 TCTTCCCAAC GTCCTGACTG GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA
      841 CAATGATTAA AGTTGAAATT AAACCATCTC AAGCCCAATT TACTACTCGT TCTGGTGTTT
      901 CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTTG TTACGTTGAT TTGGGTAATG
      961 AATATCCGGT TCTTGTCAAG ATTACTCTTG ATGAAGGTCA GCCAGCCTAT GCGCCTGGTC
     1021 TGTACACCGT TCATCTGTCC TCTTTCAAAG TTGGTCAGTT CGGTTCCCTT ATGATTGACC
     1081 GTCTGCGCCT CGTTCCGGCT AAGTAACATG GAGCAGGTCG CGGATTTCGA CACAATTTAT
     1141 CAGGCGATGA TACAAATCTC CGTTGTACTT TGTTTCGCGC TTGGTATAAT CGCTGGGGGT
     1201 CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTTAGGTTGG TGCCTTCGTA
     1261 GTGGCATTAC GTATTTTACC CGTTTAATGG AAACTTCCTC ATGAAAAAGT CTTTAGTCCT
     1321 CAAAGCCTCT GTAGCCGTTG CTACCCTCGT TCCGATGCTG TCTTTCGCTG CTGAGGGTGA
     1381 CGATCCCGCA AAAGCGGCCT TTAACTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA
     1441 TGCGTGGGCG ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTTAAGAA
     1501 ATTCACCTCG AAAGCAAGCT GATAAACCGA TACAATTAAA GGCTCCTTTT GGAGCCTTTT
     1561 TTTTTGGAGA TTTTCAACGT GAAAAAATTA TTATTCGCAA TTCCTTTAGT TGTTCCTTTC
     1621 TATTCTCACA GTGCACAGTC TGTCGTGACG CAGCCGCCCT CAGTGTCTGG GGCCCCAGGG
     1681 CAGAGGGTCA CCATCTCCTG CACTGGGAGC AGCTCCAACA TCGGGGCAGG TTATGATGTA
     1741 CACTGGTACC AGCAGCTTCC AGGAACAGCC CCCAAACTCC TCATCTATGG TAACAGCAAT
     1801 CGGCCCTCAG GGGTCCCTGA CCGATTCTCT GGCTCCAAGT CTGGCACCTC AGCCTCCCTG
     1861 GCCATCACTG GGCTCCAGGC TGAGGATGAG GCTGATTATT ACTGCCAGTC CTATGACAGC
     1921 AGCCTGAGTG GCCTTTATGT CTTCGGAACT GGGACCAAGG TCACCGTCCT AGGTCAGCCC
     1981 AAGGCCAACC CCACTGTCAC TCTGTTCCCG CCCTCCTCTG AGGAGCTCCA AGCCAACAAG
     2041 GCCACACTAG TGTGTCTGAT CAGTGACTTC TACCCGGGAG CTGTGACAGT GGCCTGGAAG
     2101 GCAGATAGCA GCCCCGTCAA GGCGGGAGTG GAGACCACCA CACCCTCCAA ACAAAGCAAC
     2161 AACAAGTACG CGGCCAGCAG CTATCTGAGC CTGACGCCTG AGCAGTGGAA GTCCCACAGA
     2221 AGCTACAGCT GCCAGGTCAC GCATGAAGGG AGCACCGTGG AGAAGACAGT GGCCCCTACA
     2281 GAATGTTCAT AATAAACCGC CTCCACCGGG CGCGCCAATT CTATTTCAAG GAGACAGTCA
     2341 TAATGAAATA CCTATTGCCT ACGGCAGCCG CTGGATTGTT ATTACTCGCG GCCCAGCCGG
     2401 CCATGGCCGA AGTTCAATTG TTAGAGTCTG GTGGCGGTCT TGTTCAGCCT GGTGGTTCTT
     2461 TACGTCTTTC TTGCGCTGCT TCCGGATTCA CTTTCTCTTC GTACGCTATG TCTTGGGTTC
     2521 GCCAAGCTCC TGGTAAAGGT TTGGAGTGGG TTTCTGCTAT CTCTGGTTCT GGTGGCAGTA
     2581 CTTACTATGC TGACTCCGTT AAAGGTCGCT TCACTATCTC TAGAGACAAC TCTAAGAATA
     2641 CTCTCTACTT GCAGATGAAC AGCTTAAGGG CTGAGGACAC TGCAGTCTAC TATTGCGCTA
     2701 AAGACTATGA AGGTACTGGT TATGCTTTCG ACATATGGGG TCAAGGTACT ATGGTCACCG
     2761 TCTCTAGTGC CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGCACCCTCC TCCAAGAGCA
     2821 CCTCTGGGGG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA
     2881 CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTCCA CACCTTCCCG GCTGTCCTAC
     2941 AGTCTAGCGG ACTCTACTCC CTCAGCAGCG TAGTGACCGT GCCCTCTTCT AGCTTGGGCA
     3001 CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA CACCAAGGTG GACAAGAAAG
     3061 TTGAGCCCAA ATCTTGTGCG GCCGCTCATC ACCACCATCA TCACTCTGCT GAACAAAAAC
     3121 TCATCTCAGA AGAGGATCTG AATGGTGCCG CAGATATCAA CGATGATCGT ATGGCTGGCG
     3181 CCGCTGAAAC TGTTGAAAGT TGTTTAGCAA AACCCCATAC AGAAAATTCA TTTACTAACG
     3241 TCTGGAAAGA CGACAAAACT TTAGATCGTT ACGCTAACTA TGAGGGTTGT CTGTGGAATG
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3301	CTACAGGCGT	TGTAGTTTGT	ACTGGTGACG	AAACTCAGTG	TTACGGTACA	TGGGTTCCTA
3361	TTGGGCTTGC	TATCCCTGAA	AATGAGGGTG	GTGGCTCTGA	GGGTGGCGGT	TCTGAGGGTG
3421	GCGGTTCTGA	GGGTGGCGGT	ACTAAACCTC	CTGAGTACGG	TGATACACCT	ATTCCGGGCT
3481	ATACTTATAT	CAACCCTCTC	GACGGCACTT	ATCCGCCTGG	TACTGAGCAA	AACCCCGCTA
3541	ATCCTAATCC	TTCTCTTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
3601	GGTTCCGAAA	TAGGCAGGGG	GCATTAACTG	TTTATACGGG	CACTGTTACT	CAAGGCACTG
3661	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC	AAAAGCCATG	TATGACGCTT
3721	ACTGGAACGG	TAAATTCAGA	GACTGCGCTT	TCCATTCTGG	CTTTAATGAA	GATCCATTCG
3781	TTTGTGAATA	TCAAGGCCAA	TCGTCTGACC	TGCCTCAACC	TCCTGTCAAT	GCTGGCGGCG
3841	GCTCTGGTGG	TGGTTCTGGT	GGCGGCTCTG	AGGGTGGTGG	CTCTGAGGGT	GGCGGTTCTG
3901	AGGGTGGCGG	CTCTGAGGGA	GGCGGTTCCG	GTGGTGGCTC	TGGTTCCGGT	GATTTTGATT
3961	ATGAAAAGAT	GGCAAACGCT	AATAAGGGGG	CTATGACCGA	AAATGCCGAT	GAAAACGCGC
4021	TACAGTCTGA	CGCTAAAGGC	AAACTTGATT	CTGTCGCTAC	TGATTACGGT	GCTGCTATCG
4081	ATGGTTTCAT	TGGTGACGTT	TCCGGCCTTG	CTAATGGTAA	TGGTGCTACT	GGTGATTTTG
4141	CTGGCTCTAA	TTCCCAAATG	GCTCAAGTCG	GTGACGGTGA	TAATTCACCT	TTAATGAATA
4201	ATTTCCGTCA	ATATTTACCT	TCCCTCCCTC	AATCGGTTGA	ATGTCGCCCT	TTTGTCTTTA
4261	GCGCTGGTAA	ACCATATGAA	TTTTCTATTG	ATTGTGACAA	AATAAACTTA	TTCCGTGGTG
4321	TCTTTGCGTT	TCTTTTATAT	GTTGCCACCT	TTATGTATGT	ATTTTCTACG	TTTGCTAACA
4381	TACTGCGTAA	TAAGGAGTCT	TAATCATGCC	AGTTCTTTTG	GGTATTCCGT	TATTATTGCG
4441	TTTCCTCGGT	TTCCTTCTGG	TAACTTTGTT	CGGCTATCTG	CTTACTTTTC	TTAAAAAGGG
4501	CTTCGGTAAG	ATAGCTATTG	CTATTTCATT	GTTTCTTGCT	CTTATTATTG	GGCTTAACTC
4561	AATTCTTGTG	GGTTATCTCT	CTGATATTAG	CGCTCAATTA	CCCTCTGACT	TTGTTCAGGG
4621	TGTTCAGTTA	ATTCTCCCGT	CTAATGCGCT	TCCCTGTTTT	TATGTTATTC	TCTCTGTAAA
4681	GGCTGCTATT	TTCATTTTTG	ACGTTAAACA	AAAAATCGTT	TCTTATTTGG	ATTGGGATAA
4741	ATAATATGGC	TGTTTATTTT	GTAACTGGCA	AATTAGGCTC	TGGAAAGACG	CTCGTTAGCG
4801	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	GGTGCAAAAT	AGCAACTAAT	CTTGATTTAA
4861	GGCTTCAAAA	CCTCCCGCAA	GTCGGGAGGT	TCGCTAAAAC	GCCTCGCGTT	CTTAGAATAC
4921	CGGATAAGCC	TTCTATATCT	GATTTGCTTG	CTATTGGGCG	CGGTAATGAT	TCCTACGATG
4981	AAAATAAAAA	CGGCTTGCTT	GTTCTCGATG	AGTGCGGTAC	TTGGTTTAAT	ACCCGTTCTT
5041	GGAATGATAA	GGAAAGACAG	CCGATTATTG	ATTGGTTTCT	ACATGCTCGT	AAATTAGGAT
5101	GGGATATTAT	TTTTCTTGTT	CAGGACTTAT	CTATTGTTGA	TAAACAGGCG	CGTTCTGCAT
5161	TAGCTGAACA	TGTTGTTTAT	TGTCGTCGTC	TGGACAGAAT	TACTTTACCT	TTTGTCGGTA
5221	CTTTATATTC	TCTTATTACT	GGCTCGAAAA	TGCCTCTGCC	TAAATTACAT	GTTGGCGTTG
5281	TTAAATATGG	CGATTCTCAA	TTAAGCCCTA	CTGTTGAGCG	TTGGCTTTAT	ACTGGTAAGA
5341	ATTTGTATAA	CGCATATGAT	ACTAAACAGG	CTTTTTCTAG	TAATTATGAT	TCCGGTGTTT
5401	ATTCTTATTT	AACGCCTTAT	TTATCACACG	GTCGGTATTT	CAAACCATTA	AATTTAGGTC
5461	AGAAGATGAA	ATTAACTAAA	ATATATTTGA	AAAAGTTTTC	TCGCGTTCTT	TGTCTTGCGA
5521	TTGGATTTGC	ATCAGCATTT	ACATATAGTT	ATATAACCCA	ACCTAAGCCG	GAGGTTAAAA
5581	AGGTAGTCTC	TCAGACCTAT	GATTTTGATA	AATTCACTAT	TGACTCTTCT	CAGCGTCTTA
5641	ATCTAAGCTA	TCGCTATGTT	TTCAAGGATT	CTAAGGGAAA	ATTAATTAAT	AGCGACGATT
5701	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTTATG	TACTGTTTCC	ATTAAAAAAG
5761	GTAATTCAAA	TGAAATTGTT	AAATGTAATT	AATTTTGTTT	TCTTGATGTT	TGTTTCATCA
5821	TCTTCTTTTG	CTCAGGTAAT	TGAAATGAAT	AATTCGCCTC	TGCGCGATTT	TGTAACTTGG
5881	TATTCAAAGC	AATCAGGCGA	ATCCGTTATT	GTTTCTCCCG	ATGTAAAAGG	TACTGTTACT
5941	GTATATTCAT	CTGACGTTAA	ACCTGAAAAT	CTACGCAATT	TCTTTATTTC	TGTTTTACGT
6001	GCTAATAATT	TTGATATGGT	TGGTTCAATT	CCTTCCATAA	TTCAGAAGTA	TAATCCAAAC
6061	AATCAGGATT	ATATTGATGA	ATTGCCATCA	TCTGATAATC	AGGAATATGA	TGATAATTCC
6121	GCTCCTTCTG	GTGGTTTCTT	TGTTCCGCAA	AATGATAATG	TTACTCAAAC	TTTTAAAATT
6181	AATAACGTTC	GGGCAAAGGA	TTTAATACGA	GTTGTCGAAT	TGTTTGTAAA	GTCTAATACT
6241	TCTAAATCCT	CAAATGTATT	ATCTATTGAC	GGCTCTAATC	TATTAGTTGT	TTCTGCACCT
6301	AAAGATATTT	TAGATAACCT	TCCTCAATTC	CTTTCTACTG	TTGATTTGCC	AACTGACCAG
6361	ATATTGATTG	AGGGTTTGAT	ATTTGAGGTT	CAGCAAGGTG	ATGCTTTAGA	TTTTTCATTT
6421	GCTGCTGGCT	CTCAGCGTGG	CACTGTTGCA	GGCGGTGTTA	ATACTGACCG	CCTCACCTCT
6481	GTTTTATCTT	CTGCTGGTGG	TTCGTTCGGT	ATTTTTAATG	GCGATGTTTT	AGGGCTATCA
6541	GTTCGCGCAT	TAAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTGCCACG	TATTCTTACG
6601	CTTTCAGGTC	AGAAGGGTTC	TATCTCTGTT	GGCCAGAATG	TCCCTTTTAT	TACTGGTCGT
6661	GTGACTGGTG	AATCTGCCAA	TGTAAATAAT	CCATTTCAGA	CGATTGAGCG	TCAAAATGTA
6721	GGTATTTCCA	TGAGCGTTTT	TCCTGTTGCA	ATGGCTGGCG	GTAATATTGT	TCTGGATATT

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6781  ACCAGCAAGG CCGATAGTTT GAGTTCTTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA
6841  AGAAGTATTG CTACAACGGT TAATTTGCGT GATGGACAGA CTCTTTTACT CGGTGGCCTC
6901  ACTGATTATA AAAACACTTC TCAAGATTCT GGCGTACCGT TCCTGTCTAA AATCCCTTTA
6961  ATCGGCCTCC TGTTTAGCTC CCGCTCTGAT TCCAACGAGG AAAGCACGTT ATACGTGCTC
7021  GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT
7081  TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT
7141  CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC
7201  TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA
7261  TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC
7321  CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG
7381  CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGAA CCACCATCAA ACAGGATTTT
7441  CGCCTGCTGG GGCAAACCAG CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG
7501  AAGGGCAATC AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGATCCAAGC
7561  TTGCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA
7621  TACATTCAAA TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT
7681  GAAAAAGGAA GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC TTTTTTGCGG
7741  CATTTTGCCT TCCTGTTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG
7801  ATCAGTTGGG CGCACGAGTG GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG
7861  AGAGTTTTCG CCCCGAAGAA CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTC
7921  ATACACTATT ATCCCGTATT GACGCCGGGC AAGAGCAACT CGGTCGCCGG GCGCGGTATT
7981  CTCAGAATGA CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA
8041  CAGTAAGAGA ATTATGCAGT GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC
8101  TTCTGACAAC GATCGGAGGA CCGAAGGAGC TAACCGCTTT TTTGCACAAC ATGGGGGATC
8161  ATGTAACTCG CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC
8221  GTGACACCAC GATGCCTGTA GCAATGCCAA CAACGTTGCG CAAACTATTA ACTGGCGAAC
8281  TACTTACTCT AGCTTCCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG
8341  GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG
8401  GTGAGCGTGG GTCTCGCGGT ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA
8461  TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG
8521  CTGAGATAGG TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA
8581  TACTTTAGAT TGATTTAAAA CTTCATTTTT AATTTAAAAG GATCTAGGTG AAGATCCTTT
8641  TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGT ACGTAAGACC
8701  CCCAAGCTTG TCGACTGAAT GGCGAATGGC GCTTTGCCTG GTTTCCGGCA CCAGAAGCGG
8761  TGCCGGAAAG CTGGCTGGAG TGCGATCTTC CTGAGGCCGA TACTGTCGTC GTCCCCTCAA
8821  ACTGGCAGAT GCACGGTTAC GATGCGCCCA TCTACACCAA CGTAACCTAT CCCATTACGG
8881  TCAATCCGCC GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG
8941  TTGATGAAAG CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTTGATGGC GTTCCTATTG
9001  GTTAAAAAAT GAGCTGATTT AACAAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT
9061  TACAATTTAA ATATTTGCTT ATACAATCTT CCTGTTTTTG GGGCTTTTCT GATTATCAAC
9121  CGGGGTACAT ATGATTGACA TGCTAGTTTT ACGATTACCG TTCATCGATT CTCTTGTTTG
9181  CTCCAGACTC TCAGGCAATG ACCTGATAGC CTTTGTAGAT CTCTCAAAAA TAGCTACCCT
9241  CTCCGGCATG AATTTATCAG CTAGAACGGT TGAATATCAT ATTGATGGTG ATTTGACTGT
9301  CTCCGGCCTT TCTCACCCTT TTGAATCTTT ACCTACACAT TACTCAGGCA TTGCATTTAA
9361  AATATATGAG GGTTCTAAAA ATTTTTATCC TTGCGTTGAA ATAAAGGCTT CTCCCGCAAA
9421  AGTATTACAG GGTCATAATG TTTTTGGTAC AACCGATTTA GCTTTATGCT CTGAGGCTTT
9481  ATTGCTTAAT TTTGCTAATT CTTTGCCTTG CCTGTATGAT TTATTGGATG TT
```

Table 22: Primers used in RACE amplification:

Heavy chain	
HuCμ-FOR (1st PCR)	5'-TGG AAG AGG CAC GTT CTT TTC TTT-3'
HuCμ-Nested (2nd PCR)	5' CTT TTC TTT GTT GCC GTT GGG GTG-3'
Kappa light chain	
HuCkFor (1st PCR)	5'-ACA CTC TCC CCT GTT GAA GCT CTT-3'
HuCkForAscI(2nd PCR)	5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA ACA CTC TCC CCT GTT GAA GCT CTT-3'
Lambda light chain	
HuClambdaFor (1st PCR)	
HuCL2-FOR	5'-TGA ACA TTC TGT AGG GGC CAC TG-3'
HuCL7-FOR	5'-AGA GCA TTC TGC AGG GGC CAC TG-3'
HuClambdaForAscI (2nd PCR)	
HuCL2-FOR-ASC	5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA TGA ACA TTC TGT AGG GGC CAC TG-3'
HuCL7-FOR-ASC	5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA AGA GCA TTC TGC AGG GGC CAC TG-3'
GeneRAcer 5' Primers provided with the kit (Invitrogen)	
5'A 1st PCR	5'CGACTGGAGCACGAGGACACTGA 3'
5'NA 2nd pCR	5'GGACACTGACATGGACTGAAGGAGTA-3'

Table 23: ONs used in Capture of kappa light chains using CJ method and *Bsm*AI

All ONs are written 5' to 3'.

REdapters (6)

ON_2OSK15O12	gggAggATggAgAcTgggTc
ON_2OSK15L12	gggAAgATggAgAcTgggTc
ON_2OSK15A17	gggAgAgTggAgAcTgAgTc
ON_2OSK15A27	gggTgccTggAgAcTgcgTc
ON_2OSK15A11	gggTggcTggAgAcTgcgTc
ON_2OSK15B3	gggAgTcTggAgAcTgggTc

Bridges (6)

kapbri1O12	gggAggATggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1L12	gggAAgATggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1A17	gggAgAgTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1A27	gggTgccTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1A11	gggTggcTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1B3	gggAgTcTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg

Extender (5' biotinylated)

kapext1bio	ccTcTgTcAcAgTgcAcAAgAcATccAgATgAcccAgTcTcc

Primers

kaPCRt1	ccTcTgTcAcA**gTgcAc**AAgAc
kapfor	5'-aca ctc tcc cct gtt gaa gct ctt-3'

Table 24: PCR program for amplification of kappa DNA

95°C	5 minutes
95°C	15 seconds
65°C	30 seconds
72°C	1 minute
72°C	7 minutes
4°C	hold

Reagents (100 ul reaction):

Template	50 ng
10x turbo PCR buffer	1x
turbo Pfu	4U
dNTPs	200 μM each
kaPCRt1	300 nM
kapfor	300 nM

Table 25: h3401-h2 captured Via CJ with BsmAI

```
! 1  2  3  4  5  6  7  8  9  10 11 12 13 14 15
! S  A  Q  D  I  Q  M  T  Q  S  P  A  T  L  S
 aGT GCA Caa gac atc cag atg acc cag tct cca gcc acc ctg tct
 ! ApaLI...                    a gcc acc ! L25,L6,L20,L2,L16,A11
 ! Extender.....................Bridge...

! 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
! V  S  P  G  E  R  A  T  L  S  C  R  A  S  Q
 gtg tct cca ggg gaa agg gcc acc ctc tcc tgc agg gcc agt cag

! 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
! S  V  S  N  N  L  A  W  Y  Q  Q  K  P  G  Q
 agt gtt agt aac aac tta gcc tgg tac cag cag aaa cct ggc cag

! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
! V  P  R  L  L  I  Y  G  A  S  T  R  A  T  D
 gtt ccc agg ctc ctc atc tat ggt gca tcc acc agg gcc act gat

! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
! I  P  A  R  F  S  G  S  G  S  G  T  D  F  T
 atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gac ttc act

! 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
! L  T  I  S  R  L  E  P  E  D  F  A  V  Y  Y
 ctc acc atc agc aga ctg gag cct gaa gat ttt gca gtg tat tac

! 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
! C  Q  R  Y  G  S  S  P  G  W  T  F  G  Q  G
 tgt cag cgg tat ggt agc tca ccg ggg tgg acg ttc ggc caa ggg

! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
! T  K  V  E  I  K  R  T  V  A  A  P  S  V  F
 acc aag gtg gaa atc aaa cga act gtg gct gca cca tct gtc ttc

! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
! I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S
 atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct

! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
! V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V
 gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
! Q  W  K  V  D  N  A  L  Q  S  G  N  S  Q  E
 cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag

! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
! S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S
 agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc

! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
```

! S T L T L S K A D Y E K H K V
agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc

! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
! Y A C E V T H Q G L S S P V T
tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg cct gtc aca

! 211 212 213 214 215 216 217 218 219 220 221 222 223
! K S F N K G E C K G E F A
aag agc ttc aac aaa gga gag tgt aag ggc gaa ttc gc.....

Table 26: h3401-d8 KAPPA captured with CJ and *Bsm*AI

```
! 1  2  3  4  5  6  7  8  9  10 11 12 13 14 15
! S  A  Q  D  I  Q  M  T  Q  S  P  A  T  L  S
  aGT GCA Caa gac atc cag atg acc cag tct cct gcc acc ctg tct
! ApaLI...Extender.....a gcc acc ! L25,L6,L20,L2,L16,A11
!                       A GCC ACC CTG TCT ! L2

! 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
! V  S  P  G  E  R  A  T  L  S  C  R  A  S  Q
  gtg tct cca ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag
! GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC !    L2

! 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
! N  L  L  S  N  L  A  W  Y  Q  Q  K  P  G  Q
  aat ctt ctc agc aac tta gcc tgg tac cag cag aaa cct ggc cag

! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
! A  P  R  L  L  I  Y  G  A  S  T  G  A  I  G
  gct ccc agg ctc ctc atc tat ggt gct tcc acc ggg gcc att ggt

! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
! I  P  A  R  F  S  G  S  G  S  G  T  E  F  T
  atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gag ttc act

! 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
! L  T  I  S  S  L  Q  S  E  D  F  A  V  Y  F
  ctc acc atc agc agc ctg cag tct gaa gat ttt gca gtg tat ttc

! 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
! C  Q  Q  Y  G  T  S  P  P  T  F  G  G  G  T
  tgt cag cag tat ggt acc tca ccg ccc act ttc ggc gga ggg acc

! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
! K  V  E  I  K  R  T  V  A  A  P  S  V  F  I
  aag gtg gag atc aaa cga act gtg gct gca cca tct gtc ttc atc

! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
! F  P  P  S  D  E  Q  L  K  S  G  T  A  S  V
  ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt

! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
! V  C  P  L  N  N  F  Y  P  R  E  A  K  V  Q
  gtg tgc ccg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
! W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S
  tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt

! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
! V  T  E  Q  D  N  K  D  S  T  Y  S  L  S  S
  gtc aca gag cag gac aac aag gac agc acc tac agc ctc agc agc
```

```
! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
! T  L  T  L  S  K  V  D  Y  E  K  H  E  V  Y
 acc ctg acg ctg agc aaa gta gac tac gag aaa cac gaa gtc tac

! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
! A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K
 gcc tgc gaa gtc acc cat cag ggc ctt agc tcg ccc gtc acg aag

! 211 212 213 214 215 216 217 218 219 220 221 222 223
! S  F  N  R  G  E  C  K  K  E  F  V
 agc ttc aac agg gga gag tgt aag aaa gaa ttc gtt t
```

Table 27: V3-23 VH framework with variegated codons shown

```
!
!                    17 18 19 20 21 22
!                    A  Q  P  A  M  A
5          5'-ctg tct gaa cG GCC cag ccG GCC atg gcc    29
           3'-gac aga ctt gc cgg gtc ggc cgg tac cgg
!            Scab.....SfiI.....
!                       NgoMI...
!                          NcoI....
10 !
!                       FR1(DP47/V3-23)----------
!                       23 24 25 26 27 28 29 30
!                        E  V  Q  L  L  E  S  G
                        gaa|gtt|CAA|TTG|tta|gag|tct|ggt|   53
15 !                    ctt|caa|gtt|aac|aat|ctc|aga|cca|
!                           | MfeI |
!
!     ----------FR1-------------------------------------
!      31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
20 !    G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A
      |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|   98
!     |ccg|cca|gaa|caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|
!
!     Sites to be varied--->      ***   ***   ***
25 !  ---FR1------------>|...CDR1.....|--FR2-----
!      46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
!       A  S  G  F  T  F  S  S  Y  A  M  S  W  V  R
      |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|  143
!     |cga|agg|cct|aag|tga|aag|aga|agc|atg|cga|tac|aga|acc|caa|gcg|
30 !    | BspEI |          | BsiWI|            |BstXI.
!
!                  Sites to be varies---> ***   *** ***
!     ------FR2------------------------>|...CDR2.....
!      61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
35 !    Q  A  P  G  K  G  L  E  W  V  S  A  I  S  G
      |CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|  188
!     |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|cga|tag|aga|cca|
! ...BstXI      |
!
40 !          ***   ***
!     .....CDR2.....|--FR3--
!      76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
!       S  G  G  S  T  Y  Y  A  D  S  V  K  G  R  F
      |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|  233
45 !  |aga|cca|ccg|tca|tga|atg|ata|cga|ctg|agg|caa|ttt|cca|gcg|aag|
!
!     -------FR3-------------------------------------
!      91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
!       T  I  S  R  D  N  S  K  N  T  L  Y  L  Q  M
50      |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|  278
!     |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|
!          | XbaI |
!
!     ---FR3-------------------------------------->|
55 !   106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!       N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  K
      |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|  323
!     |ttg|tcg|aat|tcc|cga|ctc|ctg|tga|cgt|cag|atg|ata|acg|cga|ttt|
```

```
!          |AflII |           | PstI |
!
!     .....CDR3.....|---FR4----------
!      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
5     !      D  Y  E  G  T  G  Y  A  F  D  I  W  G  Q  G
      |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|   368
!     |ctg|ata|ctt|cca|tga|cca|ata|cga|aag|ctg|tat|acc|cca|gtt|cca|
!                                  | NdeI |
!
10    !     ----------FR4-------->|
!      136 137 138 139 140 141 142
!       T  M  V  T  V  S  S
      |act|atG|GTC|ACC|gtc|tct|agt-     389
!     |tga|tac|cag|tgg|cag|aga|tca-
15    !         | BstEII |
!
!                  143 144 145 146 147 148 149 150 151 152
!                   A  S  T  K  G  P  S  V  F  P
                   gcc tcc acc aaG GGC CCa tcg GTC TTC ccc-3'   419
20    !            cgg agg tgg ttc ccg ggt agc cag aag ggg-5'
!                        Bsp120I.    BbsI...(2/2)
!                        ApaI....

(SFPRMET)  5'-ctg tct gaa cG GCC cag ccG-3'
(TOPFR1A)  5'-ctg tct gaa cG GCC cag ccG GCC atg gcc-
25              gaa|gtt|CAA|TTG|tta|gag|tct|ggt|-
               |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta-3'
(BOTFR1B)          3'-caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|-
               |cga|agg|cct|aag|tga|aag-5' ! bottom strand
(BOTFR2)   3'-acc|caa|gcg|-
30             |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|-5' ! bottom strand
(BOTFR3)   3'- a|cga|ctg|agg|caa|ttt|cca|gcg|aag|-
               |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|-
           |ttg|tcg|aat|tcc|cga|ctc|ctg|tga-5'
(F06)      5'-gC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|-
35           |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|c-3'
(BOTFR4)   3'-cga|aag|ctg|tat|acc|cca|gtt|cca|-
               |tga|tac|cag|tgg|cag|aga|tca-
                 cgg agg tgg ttc ccg ggt agc cag aag ggg-5' ! bottom strand
(BOTPRCPRIM)           3'-gg ttc ccg ggt agc cag aag ggg-5'
40    !
! CDR1 diversity
!
(ON-vgC1)  5'-|gct|TCC|GGA|ttc|act|ttc|tct|<1>|TAC|<1>|atg|<1>|-
!                      CDR1.....6859
45             |tgg|gtt|cgC|CAa|gct|ccT|GG-3'
!
!<1> stands for an equimolar mix of {ADEFGHIKLMNPQRSTVWY}; no C
!                    (this is not a sequence)
!
50  ! CDR2 diversity
!
(ON-vgC2)  5'-ggt|ttg|gag|tgg|gtt|tct|<2>|atc|<2>|<3>|-
!                     CDR2.....
             |tct|ggt|ggc|<1>|act|<1>|tat|gct|gac|tcc|gtt|aaa|gg-3'
55  !          CDR2.....
! <1> is an equimolar mixture of {ADEFGHIKLMNPQRSTVWY}; no C
! <2> is an equimolar mixture of {YRWVGS}; no ACDEFHIKLMNPQT
```

! <3> is an equimolar mixture of {PS}; no ACDEFGHIKLMNQRTVWY

Table 28: Stuffer used in VH

```
  1 TCCGGAGCTT CAGATCTGTT TGCCTTTTTG TGGGGTGGTG CAGATCGCGT TACGGAGATC
 61 GACCGACTGC TTGAGCAAAA GCCACGCTTA ACTGCTGATC AGGCATGGGA TGTTATTCGC
121 CAAACCAGTC GTCAGGATCT TAACCTGAGG CTTTTTTTAC CTACTCTGCA AGCAGCGACA
181 TCTGGTTTGA CACAGAGCGA TCCGCGTCGT CAGTTGGTAG AAACATTAAC ACGTTGGGAT
241 GGCATCAATT TGCTTAATGA TGATGGTAAA ACCTGGCAGC AGCCAGGCTC TGCCATCCTG
301 AACGTTTGGC TGACCAGTAT GTTGAAGCGT ACCGTAGTGG CTGCCGTACC TATGCCATTT
361 GATAAGTGGT ACAGCGCCAG TGGCTACGAA ACAACCCAGG ACGGCCCAAC TGGTTCGCTG
421 AATATAAGTG TTGGAGCAAA AATTTTGTAT GAGGCGGTGC AGGGAGACAA ATCACCAATC
481 CCACAGGCGG TTGATCTGTT TGCTGGGAAA CCACAGCAGG AGGTTGTGTT GGCTGCGCTG
541 GAAGATACCT GGGAGACTCT TTCCAAACGC TATGGCAATA ATGTGAGTAA CTGGAAAACA
601 CCTGCAATGG CCTTAACGTT CCGGGCAAAT AATTTCTTTG GTGTACCGCA GGCCGCAGCG
661 GAAGAAACGC GTCATCAGGC GGAGTATCAA AACCGTGGAA CAGAAAACGA TATGATTGTT
721 TTCTCACCAA CGACAAGCGA TCGTCCTGTG CTTGCCTGGG ATGTGGTCGC ACCCGGTCAG
781 AGTGGGTTTA TTGCTCCCGA TGGAACAGTT GATAAGCACT ATGAAGATCA GCTGAAAATG
841 TACGAAAATT TTGGCCGTAA GTCGCTCTGG TTAACGAAGC AGGATGTGGA GGCGCATAAG
901 GAGTCGTCTA GA
```

Table 29: DNA sequence of pCES5

```
! pCES5  6680  bases = pCes4 with stuffers in CDR1-2 and CDR3 2000.12.13
!
! Ngene =  6680
! Useful REs (cut MAnoLI fewer than 3 times) 2000.06.05
!
! Non-cutters
!Acc65I Ggtacc      AfeI AGCgct        AvrII Cctagg
!BsaBI GATNNnnatc   BsiWI Cgtacg       BsmFI Nnnnnnnnnnnnnnngtccc
!BsrGI Tgtaca       BstAPI GCANNNNntgc  BstBI TTcgaa
!BstZ17I GTAtac     BtrI CACgtg        Ecl136I GAGctc
!EcoRV GATatc       FseI GGCCGGcc      KpnI GGTACc
!MscI TGGcca        NruI TCGcga        NsiI ATGCAt
!PacI TTAATtaa      PmeI GTTTaaac      PmlI CACgtg
!PpuMI RGgwccy      PshAI GACNNnngtc   SacI GAGCTc
!SacII CCGCgg       SbfI CCTGCAgg      SexAI Accwggt
!SgfI GCGATcgc      SnaBI TACgta       SpeI Actagt
!SphI GCATGc        Sse8387I CCTGCAgg  StuI AGGcct
!SwaI ATTTaaat      XmaI Cccggg
!
! cutters
! Enzymes that cut more than    3 times.
!AlwNI CAGNNNctg            5
!BsgI ctgcac              4
!BsrFI Rccggy              5
!EarI CTCTTCNnnn           4
!FauI nNNNNNNGCGGG         10
!
! Enzymes that cut from 1 to    3 times.
!
!EcoO109I RGgnccy          3     7  2636  4208
!BssSI Ctcgtg              1    12
!-"-  Cacgag               1  1703
!BspHI Tcatga              3    43  148  1156
```

!AatII GACGTc 1 65
!BciVI GTATCCNNNNNN 2 140 1667
!Eco57I CTGAAG 1 301
!-"- cttcag 2 1349
!AvaI Cycgrg 3 319 2347 6137
!BsiHKAI GWGCWc 3 401 2321 4245
!HgiAI GWGCWc 3 401 2321 4245
!BcgI gcannnnnntcg 1 461
!ScaI AGTact 1 505
!PvuI CGATcg 3 616 3598 5926
!FspI TGCgca 2 763 5946
!BglI GCCNNNNnggc 3 864 2771 5952
!BpmI CTGGAG 1 898
!-"- ctccag 1 4413
!BsaI GGTCTCNnnnn 1 916
!AhdI GACNNNnngtc 1 983
!Eam1105I GACNNNnngtc 1 983
!DrdI GACNNNNnngtc 3 1768 6197 6579
!SapI gaagagc 1 1998
!PvuII CAGctg 3 2054 3689 5896
!PflMI CCANNNNntgg 3 2233 3943 3991
!HindIII Aagctt 1 2235
!ApaLI Gtgcac 1 2321
!BspMI Nnnnnnnnngcaggt 1 2328
!-"- ACCTGCNNNNn 2 3460
!PstI CTGCAg 1 2335
!AccI GTmkac 2 2341 2611
!HincII GTYrac 2 2341 3730
!SalI Gtcgac 1 2341
!TliI Ctcgag 1 2347
!XhoI Ctcgag 1 2347
!BbsI gtcttc 2 2383 4219
!BlpI GCtnagc 1 2580
!EspI GCtnagc 1 2580
!SgrAI CRccggyg 1 2648
!AgeI Accggt 2 2649 4302

!AscI GGcgcgcc 1 2689
!BssHII Gcgcgc 1 2690
!SfiI GGCCNNNNnggcc 1 2770
!NaeI GCCggc 2 2776 6349
!NgoMIV Gccggc 2 2776 6349
!BtgI Ccrygg 3 2781 3553 5712
!DsaI Ccrygg 3 2781 3553 5712
!NcoI Ccatgg 1 2781
!StyI Ccwwgg 3 2781 4205 4472
!MfeI Caattg 1 2795
!BspEI Tccgga 1 2861
!BglII Agatct 1 2872
!BclI Tgatca 1 2956
!Bsu36I CCtnagg 3 3004 4143 4373
!XcmI CCANNNNNnnnntgg 1 3215
!MluI Acgcgt 1 3527
!HpaI GTTaac 1 3730
!XbaI Tctaga 1 3767
!
!AflII Cttaag 1 3811
!BsmI NGcattc 1 3821
!-"- GAATGCN 1 4695
!RsrII CGgwccg 1 3827
!NheI Gctagc 1 4166
!BstEII Ggtnacc 1 4182
!BsmBI CGTCTCNnnnn 2 4188 6625
!-"- Nnnnnngagacg 1 6673
!ApaI GGGCCc 1 4209
!BanII GRGCYc 3 4209 4492 6319
!Bsp120I Gggccc 1 4209
!PspOMI Gggccc 1 4209
!BseRI NNnnnnnnnnctcctc 1 4226
!-"- GAGGAGNNNNNNNNN 1 4957
!EcoNI CCTNNnnnagg 1 4278
!PflFI GACNnngtc 1 4308
!Tth111I GACNnngtc 1 4308

```
!KasI Ggcgcc              2   4327 5967
!BstXI CCANNNNNntgg           1   4415
!NotI GCggccgc            1   4507
!EagI Cggccg              1   4508
!BamHI Ggatcc             1   5169
!BspDI ATcgat             1   5476
!NdeI CAtatg              1   5672
!EcoRI Gaattc             1   5806
!PsiI TTAtaa              1   6118
!DraIII CACNNNgtg             1   6243
!BsaAI YACgtr             1   6246
!-------------------------------------------------------------
   1   gacgaaaggg cCTCGTGata cgcctatttt tataggttaa tgtcatgata ataatggttt
!                BssSI.(1/2)
  61   cttaGACGTC aggtggcact tttcggggaa atgtgcgcgg aacccctatt tgtttatttt
!        AatII.
 121   tctaaataca ttcaaatatG TATCCgctca tgagacaata accctgataa atgcttcaat
!                    BciVI..(1 of 2)
 181   aatattgaaa aaggaagagt
! Base #  201 to 1061 = ApR gene from pUC119 with some RE sites removed
!
!       1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
!      fM  S  I  Q  H  F  R  V  A  L  I  P  F  F  A
 201   atg agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg
!
!       16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
!       A  F  C  L  P  V  F  A  H  P  E  T  L  V  K
 246   gca ttt tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa
!
!       31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
!       V  K  D  A  E  D  Q  L  G  A  R  V  G  Y  I
 291   gta aaa gat gct gaa gat cag ttg ggt gcc cga gtg ggt tac atc
!
!       46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
!       E  L  D  L  N  S  G  K  I  L  E  S  F  R  P
 336   gaa ctg gat ctc aac agc ggt aag atc ctt gag agt ttt cgc ccc
```

```
!
!      61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
!      E  E  R  F  P  M  M  S  T  F  K  V  L  L  C
  381  gaa gaa cgt ttt cca atg atg agc act ttt aaa gtt ctg cta tgt
!
!      76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
!      G  A  V  L  S  R  I  D  A  G  Q  E  Q  L  G
  426  ggc gcg gta tta tcc cgt att gac gcc ggg caa gaG CAa ctc ggT
!                                  BcgI.....
!
!      91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
!      R  R  I  H  Y  S  Q  N  D  L  V  E  Y  S  P
  471  CGc cgc ata cac tat tct cag aat gac ttg gtt gAG TAC Tca cca
!..BcgI.....                        ScaI....
!
!      106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!      V  T  E  K  H  L  T  D  G  M  T  V  R  E  L
  516  gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta
!
!      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!      C  S  A  A  I  T  M  S  D  N  T  A  A  N  L
  561  tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta
!
!      136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!      L  L  T  T  I  G  G  P  K  E  L  T  A  F  L
  606  ctt ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg
!            PvuI.... (1/2)
!
!      151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!      H  N  M  G  D  H  V  T  R  L  D  R  W  E  P
  651  cac aac atg ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg
!
!      166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!      E  L  N  E  A  I  P  N  D  E  R  D  T  T  M
  696  gag ctg aat gaa gcc ata cca aac gac gag cgt gac acc acg atg
!
```

```
!       181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!       P  V  A  M  A  T  T  L  R  K  L  L  T  G  E
  741   cct gta GCA ATG gca aca acg tTG CGC Aaa cta tta act ggc gaa
!           BsrDI..(1/2)       FspI.... (1/2)
!
!       196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!       L  L  T  L  A  S  R  Q  Q  L  I  D  W  M  E
  786   cta ctt act cta gct tcc cgg caa caa tta ata gac tgg atg gag
!
!       211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!       A  D  K  V  A  G  P  L  L  R  S  A  L  P  A
  831   gcg gat aaa gtt gca gga cca ctt ctg cgc tcg gcc ctt ccg gct
!
!       226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!       G  W  F  I  A  D  K  S  G  A  G  E  R  G  S
  876   ggc tgg ttt att gct gat aaa tCT GGA Gcc ggt gag cgt gGG TCT
!                          BpmI....(1/2)       BsaI....
!
!       241 242 243 244 245 246 247 248 249 250 251 252 253 254 255.
!       R  G  I  I  A  A  L  G  P  D  G  K  P  S  R
  921   Cgc ggt atC ATT GCa gca ctg ggg cca gat ggt aag ccc tcc cgt
! BsaI.....      BsrDI...(2/2)
!
!       256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!       I  V  V  I  Y  T  T  G  S  Q  A  T  M  D  E
  966   atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa
!                    AhdI.....
!
!       271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!       R  N  R  Q  I  A  E  I  G  A  S  L  I  K  H
 1011   cga aat aga cag atc gct gag ata ggt gcc tca ctg att aag cat
!
!       286 287
!       W  .
 1056   tgg taa
 1062                                  ctgtcagac caagtttact
```

```
      1081   catatatact ttagattgat ttaaaacttc atttttaatt taaaaggatc taggtgaaga
      1141   tccttttga taatctcatg accaaaatcc cttaacgtga gttttcgttc cactgagcgt
      1201   cagaccccgt agaaaagatc aaaggatctt cttgagatcc ttttttctg cgcgtaatct
      1261   gctgcttgca aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc
 5    1321   taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc
      1381   ttctagtgta gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc
      1441   tcgctctgct aatcctgtta ccagtggctg ctgccagtgg cgataagtcg tgtcttaccg
      1501   ggttggactc aagacgatag ttaccggata aggcgcagcg gtcgggctga acgggggttt
      1561   cgtgcataca gcccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg
10    1621   agcattgaga aagcgccacg cttcccgaag ggagaaaggc ggacagGTAT CCggtaagcg
      !                                    BciVI.. (2 of 2)
      1681   gcagggtcgg aacaggagag cgCACGAGgg agcttccagg gggaaacgcc tggtatcttt
      !                 BssSI.(2/2)
      1741   atagtcctgt cgggtttcgc cacctctgac ttgagcgtcg atttttgtga tgctcgtcag
15    1801   ggggggcgga cctatggaaa aacgccagca acgcggcctt tttacggttc ctggcctttt
      1861   gctggccttt tgctcACATG Ttctttcctg cgttatcccc tgattctgtg gataaccgta
      !              PciI...
      1921   ttaccgcctt tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcgagt
      1981   cagtgagcga ggaagcgGAA GAGCgcccaa tacgcaaacc gcctctcccc gcgcgttggc
20    !              SapI....
      2041   cgattcatta atgCAGCTGg cacgacaggt ttcccgactg gaaagcgggc agtgagcgca
      !           PvuII.(1/3)
      2101   acgcaatTAA TGTgagttag ctcactcatt aggcacccca ggcTTTACAc tttatgcttc
      !        ..-35..      Plac              ..-10.
25    2161   cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacaCAGGA AACAGCTATG
      !                                    M13Rev_seq_primer
      2221   ACcatgatta cgCCAAGCTT TGGagccttt tttttggaga ttttcaac
      !           PflMI.....
      !            Hind3.
30    ! signal::linker::CLight
      !
      !      1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
      !     fM  K  K  L  L  F  A  I  P  L  V  V  P  F  Y
      2269   gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
35    !
      !                Linker..... End of FR4
```

```
!       16 17 18 19   20 21 22 23 24 25 26 27 28 29 30
!       S  H  S  A    Q  V  Q  L  Q  V  D  L  E  I  K
 2314   tct cac aGT GCA  Cag gtc caa CTG CAG GTC GAC CTC GAG atc aaa
!          ApaLI.....      PstI...     XhoI...
!                          BspMI...
!                             SalI...
!                             AccI...(1/2)
!                             HincII.(1/2)
!
! Vlight domains could be cloned in as ApaLI-XhoI fragments.
! VL-CL(kappa) segments can be cloned in as ApaLI-AscI fragments. <-------
!
!       Ckappa-----------------------------------
!       31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
!       R  G  T  V  A  A  P  S  V  F  I  F  P  P  S
 2359   cgt gga act gtg gct gca cca tct GTC TTC atc ttc ccg cca tct
!                          BbsI...(1/2)
!
!       46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
!       D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L
 2404   gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg
!
!       61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
!       N  N  F  Y  P  R  E  A  K  V  Q  W  K  V  D
 2449   aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat
!
!       76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
!       N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q
 2494   aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag
!
!       91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
!       D  S  K  D  S  T  Y  S  L  S  S  T  L  T  L
 2539   gac agc aag gac agc acc tac agc ctc agc agc acc ctg acG CTG
!                                          EspI...
!
!      106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
```

```
!      S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V
 2584    AGC aaa gca gac tac gag aaa cac aaa GTC TAC gcc tgc gaa gtc
! ...EspI....                          AccI...(2/2)
!
 5  !      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!       T  H  Q  G  L  S  S  P  V  T  K  S  F  N  R
 2629    acc cat cag ggc ctg agt tcA CCG GTg aca aag agc ttc aac agg
!                        AgeI....(1/2)
!
10 !      136 137 138 139 140
!       G  E  C  .  .
 2674    gga gag tgt taa taa   GG CGCGCCaatt
!                      AscI.....
!                      BssHII.
15 !
 2701    ctatttcaag gagacagtca ta
!
! PelB::3-23(stuffed)::CH1::III fusion gene
!
20 !      1  2  3  4  5  6  7  8  9  10 11 12 13 14 15
!     M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L
 2723   atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc
!
!-----------------------------------
25 !
!    16 17 18 19 20 21 22
!    A  A  Q  P  A  M  A
 2768 gcG GCC cag ccG GCC atg gcc
!     SfiI.............
30 !          NgoMIV..(1/2)
!              NcoI....
!
!                      FR1(DP47/V3-23)----------
!                      23 24 25 26 27 28 29 30
35 !                    E  V  Q  L  L  E  S  G
 2789                   gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
```

```
!                          | MfeI |
!
!     ----------FR1----------------------------------
!      31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
!      G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A
 2813 |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
!
!     ---FR1----
!     46 47 48
!      A  S  G
 2858 |gct|TCC|GGA|
!       | BspEI |
!
!       Stuffer for CDR1, FR2, and CDR2---------------------------->
!       There are no stop codons in this stuffer.
 2867                                    gcttcAGATC Tgtttgcctt
!                                         BglII..
 2887   tttgtggggt ggtgcagatc gcgttacgga gatcgaccga ctgcttgagc aaaagccacg
 2947   cttaactgcT GATCAggcat gggatgttat tcgccaaacc agtcgtcagg atcttaacct
!           BclI...
 3007   gaggcttttt ttacctactc tgcaagcagc gacatctggt ttgacacaga gcgatccgcg
 3067   tcgtcagttg gtagaaacat taacacgttg ggatggcatc aatttgctta atgatgatgg
 3127   taaaacctgg cagcagccag gctctgccat cctgaacgtt tggctgacca gtatgttgaa
 3187   gcgtaccgta gtggctgccg tacctatgCC Atttgataag TGGtacagcg ccagtggcta
!                        XcmI.....
 3247   cgaaacaacc caggacggcc caactggttc gctgaatata agtgttggag caaaaatttt
 3307   gtatgaggcg gtgcagggag acaaatcacc aatcccacag gcggttgatc tgtttgctgg
 3367   gaaaccacag caggaggttg tgttggctgc gctggaagat acctgggaga ctctttccaa
 3427   acgctatggc aataatgtga gtaactggaa aacacctgca atggccttaa cgttccgggc
 3487   aaataatttc tttggtgtac cgcaggccgc agcggaagaa ACGCGTcatc aggcggagta
!                                    MluI..
 3547   tcaaaaccgt ggaacagaaa acgatatgat tgttttctca ccaacgacaa gcgatcgtcc
 3607   tgtgcttgcc tgggatgtgg tcgcacccgg tcagagtggg tttattgctc ccgatggaac
 3667   agttgataag cactatgaag atcagctgaa aatgtacgaa aattttggcc gtaagtcgct
!                 PvuII.
 3727   ctgGTTAACg aagcaggatg tggaggcgca taaggagtcg
```

```
!          HpaI..
!          HincII(2/2)
!
!     ------FR3-----------------------------------------
!            4  5  6  7  8  9  10 11 12 13 14 15 16
!            93 94 95 96 97 98 99 100 101 102 103 104 105
!            S  R  D  N  S  K  N  T  L  Y  L  Q  M
 3767         |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
!           | XbaI |
!
!     ---FR3---------------------------------------->|
!       17  18  19  20
!      106 !07 108 109
!       N  S  L  s  l  s  i  r  s  g
 3806  |aac|agC|TTA|AG t ctg agc att CGG TCC G
!          |AflII |             RsrII..
!
!          q  h  s  p  t  .
 3834     gg caa cat tct cca aac tga   ccagacga cacaaacggc
 3872     ttacgctaaa tcccgcgcat gggatggtaa agaggtggcg tctttgctgg cctggactca
 3932     tcagatgaag gccaaaaatt ggcaggagtg gacacagcag gcagcgaaac aagcactgac
 3992     catcaactgg tactatgctg atgtaaacgg caatattggt tatgttcata ctggtgctta
 4052     tccagatcgt caatcaggcc atgatccgcg attacccgtt cctggtacgg gaaaatggga
 4112     ctggaaaggg ctattgcctt ttgaaatgaa ccctaaggtg tataaccccc ag
 4164          aa GCTAGC ctgcggcttc
!              NheI..
!
 4182     G|GTC|ACC|                              gtc tca agc
!       | BstEII |
!

!        136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!         A  S  T  K  G  P  S  V  F  P  L  A  P  S  S
 4198     gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc
!
!        151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
```

```
!      K S T S G G T A A L G C L V K
 4243   aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag
!
!      166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!      D Y F P E P V T V S W N S G A
 4288   gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc
!
!      181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!      L T S G V H T F P A V L Q S S
 4333   ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tcc tca
!
!      196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!      G L Y S L S S V V T V P S S S
 4378   gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc agc
!
!     211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!      L G T Q T Y I C N V N H K P S
 4423   ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc
!
!      226 227 228 229 230 231 232 233 234 235 236 237 238
!      N T K V D K K V E P K S C
 4468   aac act aag gtg gac aaG AAA GTT GAG CCC AAA TCT TGT
!                 ON-TQHCforw.....
!
!                    Poly His linker
!            139 140 141 142 143 144 145 146 147 148 149 150
!            A A A H H H H H H G A A
 4507         GCG GCC GCa cat cat cat cac cat cac ggg gcc gca
!            NotI.....
!            EagI....
!
!    151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!    E Q K L I S E E D L N G A A .
 4543  gaa caa aaa ctc atc tca gaa gag gat ctg aat ggg gcc gca tag
!
!    Mature III---------------------------------------->...
```

```
!     166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!     T V E S C L A K P H T E N S F
 4588  act gtt gaa agt tgt tta gca aaa cct cat aca gaa aat tca ttt
!
!     181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!     T N V W K D D K T L D R Y A N
 4633  act aac gtc tgg aaa gac gac aaa act tta gat cgt tac gct aac
!
!     196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!     Y E G C L W N A T G V V V C T
 4678  tat gag ggc tgt ctg tgG AAT GCt aca ggc gtt gtg gtt tgt act
!                  BsmI....
!
!     211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!     G D E T Q C Y G T W V P I G L
 4723  ggt gac gaa act cag tgt tac ggt aca tgg gtt cct att ggg ctt
!
!     226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!     A I P E N E G G G S E G G G S
 4768  gct atc cct gaa aat gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!     241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!     E G G G S E G G G T K P P E Y
 4813  gag ggt ggc ggt tct gag ggt ggc ggt act aaa cct cct gag tac
!
!     256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!     G D T P I P G Y T Y I N P L D
 4858  ggt gat aca cct att ccg ggc tat act tat atc aac cct ctc gac
!
!     271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!     G T Y P P G T E Q N P A N P N
 4903  ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct aat
!
```

```
!     286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!      P  S  L  E  E  S  Q  P  L  N  T  F  M  F  Q
 4948  cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag
!           BseRI..(2/2)
!
!     301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
!      N  N  R  F  R  N  R  Q  G  A  L  T  V  Y  T
 4993  aat aat agg ttc cga aat agg cag ggt gca tta act gtt tat acg
!
!     316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!      G  T  V  T  Q  G  T  D  P  V  K  T  Y  Y  Q
 5038  ggc act gtt act caa ggc act gac ccc gtt aaa act tat tac cag
!
!     331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!      Y  T  P  V  S  S  K  A  M  Y  D  A  Y  W  N
 5083  tac act cct gta tca tca aaa gcc atg tat gac gct tac tgg aac
!
!     346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!      G  K  F  R  D  C  A  F  H  S  G  F  N  E  D
 5128  ggt aaa ttc aga gac tgc gct ttc cat tct ggc ttt aat gaG GAT
!                                              BamHI..
!
!     361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!      P  F  V  C  E  Y  Q  G  Q  S  S  D  L  P  Q
 5173  CCa ttc gtt tgt gaa tat caa ggc caa tcg tct gAC CTG Cct caa
! BamHI...                                 BspMI...(2/2)
!
!     376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!      P  P  V  N  A  G  G  G  S  G  G  G  S  G  G
 5218  cct cct gtc aat gct ggc ggc ggc tct ggt ggt ggt tct ggt ggc
!
!     391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!      G  S  E  G  G  G  S  E  G  G  G  S  E  G  G
 5263  ggc tct gag ggt ggc ggc tct gag ggt ggc ggt tct gag ggt ggc
!
!     406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
```

```
!    G S E G G G S G G G S G S G D
 5308  ggc tct gag ggt ggc ggt tcc ggt ggc ggc tcc ggt tcc ggt gat
!
!    421 422 423 424 425 426 427 428 429 430 431 432 433 434 435
!    F D Y E K M A N A N K G A M T
 5353  ttt gat tat gaa aaa atg gca aac gct aat aag ggg gct atg acc
!
!    436 437 438 439 440 441 442 443 444 445 446 447 448 449 450
!    E N A D E N A L Q S D A K G K
 5398  gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc aaa
!
!    451 452 453 454 455 456 457 458 459 460 461 462 463 464 465
!    L D S V A T D Y G A A I D G F
 5443  ctt gat tct gtc gct act gat tac ggt gct gct ATC GAT ggt ttc
!                                  BspDI..
!
!    466 467 468 469 470 471 472 473 474 475 476 477 478 479 480
!    I G D V S G L A N G N G A T G
 5488  att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt
!
!    481 482 483 484 485 486 487 488 489 490 491 492 493 494 495
!    D F A G S N S Q M A Q V G D G
 5533  gat ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt
!
!    496 497 498 499 500 501 502 503 504 505 506 507 508 509 510
!    D N S P L M N N F R Q Y L P S
 5578  gat aat tca cct tta atg aat aat ttc cgt caa tat tta cct tct
!
!    511 512 513 514 515 516 517 518 519 520 521 522 523 524 525
!    L P Q S V E C R P Y V F G A G
 5623  ttg cct cag tcg gtt gaa tgt cgc cct tat gtc ttt ggc gct ggt
!
!    526 527 528 529 530 531 532 533 534 535 536 537 538 539 540
!    K P Y E F S I D C D K I N L F
 5668  aaa cCA TAT Gaa ttt tct att gat tgt gac aaa ata aac tta ttc
!       NdeI....
```

```
!
!     541 542 543 544 545 546 547 548 549 550 551 552 553 554 555
!      R  G  V  F  A  F  L  L  Y  V  A  T  F  M  Y
 5713  cgt ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat
!
!     556 557 558 559 560 561 562 563 564 565 566 567 568 569 570
!      V  F  S  T  F  A  N  I  L  R  N  K  E  S  .
 5758  gta ttt tcg acg ttt gct aac ata ctg cgt aat aag gag tct taa
!
!     571
!
 5803  taa GAATTC
!        EcoRI.
 5812    actggccgt cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc caacttaatc
 5871    gccttgcagc acatcccccc ttcgccagct ggcgtaatag cgaagaggcc cgcacCGATC
!                                                          PvuI..
 5931    Gcccttccca acagtTGCGC Agcctgaatg gcgaatGGCG CCtgatgcgg tattttctcc
! ...PvuI... (3/3)       FspI... (2/2)       KasI...(2/2)
 5991    ttacgcatct gtgcggtatt tcacaccgca tataaattgt aaacgttaat attttgttaa
 6051    aattcgcgtt aaatttttgt taaatcagct catttttaa ccaataggcc gaaatcggca
 6111    aaatcccTTA TAAatcaaaa gaatagcccg agatagggtt gagtgttgtt ccagtttgga
!          PsiI...
 6171    acaagagtcc actattaaag aacgtggact ccaacgtcaa agggcgaaaa accgtctatc
 6231    agggcgatgg ccCACtacGT Gaaccatcac ccaaatcaag ttttttgggg tcgaggtgcc
!              DraIII....
6291 gtaaagcact aaatcggaac cctaaaggga gcccccgatt tagagcttga cggggaaaGC
!                                                    NgoMIV..
 6351    CGGCgaacgt ggcgagaaag gaagggaaga aagcgaaagg agcgggcgct agggcgctgg
!   ..NgoMIV.(2/2)
 6411    caagtgtagc ggtcacgctg cgcgtaacca ccacacccgc cgcgcttaat gcgccgctac
 6471    agggcgcgta ctatggttgc tttgacgggt gcagtctcag tacaatctgc tctgatgccg
 6531    catagttaag ccagccccga cacccgccaa cacccgctga cgcgccctga cgggcttgtc
 6591    tgctcccggc atccgcttac agacaagctg tgaccgtctc cgggagctgc atgtgtcaga
 6651    ggttttcacc gtcatcaccg aaacgcgcga
```

Table 30: Oligonucleotides used to clone CDR1/2 diversity

All sequences are 5' to 3'.

1) ON_CD1Bsp, 30 bases

A c c T c A c T g g c T T c c g g A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

T T c A c T T T c T c T
19 20 21 22 23 24 25 26 27 28 29 30

2) ON_Br12, 42 bases

A g A A A c c c A c T c c A A A c c
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

T T T A c c A g g A g c T T g g c g
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

A A c c c A
37 38 39 40 41 42

3) ON_CD2Xba, 51 bases

g g A A g g c A g T g A T c T A g A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

g A T A g T g A A g c g A c c T T T
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

A A c g g A g T c A g c A T A
37 38 39 40 41 42 43 44 45 46 47 48 49 50 51

4) ON_BotXba, 23 bases

g g A A g g c A g T g A T c T A g A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

g A T A g
19 20 21 22 23

Table 31: Bridge/Extender Oligonucleotides

ON_LamlaB7(rc)	.....GTGCTGACTCAGCCACCCTC.	20
ON_Lam2aB7(rc)	.....GCCCTGACTCAGCCTGCCTC.	20
ON_Lam31B7(rc)	.....GAGCTGACTCAGG.ACCCTGC	20
ON_Lam3rB7(rc)	.....GAGCTGACTCAGCCACCCTC.	20
ON_LamHf1cBrg(rc)	CCTCGACAGCGAAGTGCACAGAGCGTCTTGACTCAGCC.....	38
ON_LamHf1cExt	CCTCGACAGCGAAGTGCACAGAGCGTCTTG.....	30
ON_LamHf2b2Brg(rc)	CCTCGACAGCGAAGTGCACAGAGCGCTTTGACTCAGCC.....	38
ON_LamHf2b2Ext	CCTCGACAGCGAAGTGCACAGAGCGCTTTG.....	30
ON_LamHf2dBrg(rc)	CCTCGACAGCTAAGTGCACAGAGCGCTTTGACTCAGCC.....	38
ON_LamHf2dExt	CCTCGACAGCGAAGTGCACAGAGCGCTTTG.....	30
ON_LamHf31Brg(rc)	CCTCGACAGCGAAGTGCACAGAGCGAATTGACTCAGCC.....	38
ON_LamHf31Ext	CCTCGACAGCGAAGTGCACAGAGCGAATTG.....	30
ON_LamHf3rBrg(rc)	CCTCGACAGCGAAGTGCACAGTACGAATTGACTCAGCC.....	38
ON_LamHf3rExt	CCTCGACAGCGAAGTGCACAGTACGAATTG.....	30
ON_lamPlePCR	CCTCGACAGCGAAGTGCACAG.....	21
Consensus		

Table 32: Oligonucleotides used to make SSDNA locally double-stranded

Adapters (8)	
H43HF3.1-02#1	5'-cc gtg tat tac tgt gcg aga g-3'
H43.77.97.1-03#2	5'-ct gtg tat tac tgt gcg aga g-3'
H43.77.97.323#22	5'-cc gta tat tac tgt gcg aaa g-3'
H43.77.97.330#23	5'-ct gtg tat tac tgt gcg aaa g-3'
H43.77.97.439#44	5'-ct gtg tat tac tgt gcg aga c-3'
H43.77.97.551#48	5'-cc atg tat tac tgt gcg aga c-3'

Table 33: Bridge/extender pairs

Bridges (2)

H43.XABr1

5'ggtgtagtgaTCTAGtgacaactctaagaatactctctacttgcagatgaacagC
TTtAGggctgaggacaCTGCAGtctactattgtgcgaga-3'

H43.XABr2

5'ggtgtagtgaTCTAGtgacaactctaagaatactctctacttgcagatgaacagC
TTtAGggctgagga**c**aCTGCAGtctactattgtgcgaaa-3'

Extender

H43.XAExt

5'ATAgTAgAcTgcAgTgTccTcAgcccTTAAgcTgTTcATcTgcAAgTAgAgAgTA
TTcTTAgAgTTgTcTcTAgATcAcTAcAcc-3'

Table 34: PCR primers

<u>Primers</u>	
H43.XAPCR2	gactgggTgTAgTgATcTAg
Hucmnest	cttttctttgttgccgttggggtg

Table 35: PCR program for amplification of heavy chain CDR3 DNA

95 degrees C	5 minutes	
95 degrees C	20 seconds	
60 degrees C	30 seconds	repeat 20x
72 degrees C	1 minute	
72 degrees C	7 minutes	
4 degrees C	hold	

Reagents (100 ul reaction):

Template	5ul ligation mix
10x PCR buffer	1x
Taq	5U
dNTPs	200 uM each
$MgCl_2$	2mM
H43.XAPCR2-biotin	400 nM
Hucmnest	200 nM

```
! Table 36: Annotated sequence of CJR DY3F7(CJR-A05)  10251 bases
!
! Non-cutters
!
!BclI Tgatca          BsiWI Cgtacg         BssSI Cacgag
!BstZ17I GTAtac       BtrI CACgtg          EcoRV GATatc
!FseI GGCCGGcc        HpaI GTTaac          MluI Acgcgt
!PmeI GTTTaaac        PmlI CACgtg          PpuMI RGgwccy
!RsrII CGgwccg        SapI GCTCTTC         SexAI Accwggt
!SgfI GCGATcgc        SgrAI CRccggyg       SphI GCATGc
!StuI AGGcct          XmaI Cccggg
!
! cutters
!
! Enzymes that cut from 1 to 4 times and other features
!
!End of genes II and X                829
!Start gene V                         843
!BsrGI Tgtaca                  1     1021
!BspMI Nnnnnnnnngcaggt          3     1104  5997  9183
!-"-   ACCTGCNNNNn              1     2281
!End of gene V                        1106
!Start gene VII                       1108
!BsaBI GATNNnnatc               2     1149  3967
!Start gene IX                        1208
!End gene VII                         1211
!SnaBI TACgta                  2     1268  7133
!BspHI Tcatga                  3     1299  6085  7093
!Start gene VIII                      1301
!End gene IX                          1304
!End gene VIII                        1522
!Start gene III                       1578
!EagI Cggccg                   2     1630  8905
!XbaI Tctaga                   2     1643  8436
!KasI Ggcgcc                   4     1650  8724  9039  9120
!BsmI GAATGCN                  2     1769  9065
!BseRI GAGGAGNNNNNNNNNN        2     2031  8516
!-"-   NNnnnnnnnnctcctc        2     7603  8623
!AlwNI CAGNNNctg               3     2210  8072  8182
!BspDI ATcgat                  2     2520  9883
!NdeI CAtatg                   3     2716  3796  9847
!End gene III                         2846
!Start gene VI                        2848
!AfeI AGCgct                   1     3032
!End gene VI                          3187
!Start gene I                         3189
!EarI CTCTTCNnnn               2     4067  9274
!-"-  Nnnnngaagag              2     6126  8953
!PacI TTAATtaa                 1     4125
!Start gene IV                        4213
!End gene I                           4235
!BsmFI Nnnnnnnnnnnnnnngtccc    2     5068  9515
!MscI TGGcca                   3     5073  7597  9160
!PsiI TTAtaa                   2     5349  5837
!End gene IV                          5493
!Start ori                            5494
!NgoMIV Gccggc                 3     5606  8213  9315
!BanII GRGCYc                  4     5636  8080  8606  8889
!DraIII CACNNNgtg              1     5709
!DrdI GACNNNNnngtc             1     5752
!AvaI Cycgrg                   2     5818  7240
!PvuII CAGctg                  1     5953
```

!BsmBI CGTCTCNnnnn	3	5964	8585	9271
!End ori region		5993		
!BamHI Ggatcc	1	5994		
!HindIII Aagctt	3	6000	7147	7384
!BciVI GTATCCNNNNNN	1	6077		
!Start bla		6138		
!Eco57I CTGAAG	2	6238	7716	
!SpeI Actagt	1	6257		
!BcgI gcannnnnntcg	1	6398		
!ScaI AGTact	1	6442		
!PvuI CGATcg	1	6553		
!FspI TGCgca	1	6700		
!BglI GCCNNNNnggc	3	6801	8208	8976
!BsaI GGTCTCNnnnn	1	6853		
!AhdI GACNNNnngtc	1	6920		
!Eam1105I GACNNNnngtc	1	6920		
!End bla		6998		
!AccI GTmkac	2	7153	8048	
!HincII GTYrac	1	7153		
!SalI Gtcgac	1	7153		
!XhoI Ctcgag	1	7240		
!Start PlacZ region		7246		
!End PlacZ region		7381		
!PflMI CCANNNNntgg	1	7382		
!RBS1		7405		
!start M13-iii signal seq for LC		7418		
!ApaLI Gtgcac	1	7470		
!end M13-iii signal seq		7471		
!Start light chain kappa L20:JK1		7472		
!PflFI GACNnngtc	3	7489	8705	9099
!SbfI CCTGCAgg	1	7542		
!PstI CTGCAg	1	7543		
!KpnI GGTACc	1	7581		
!XcmI CCANNNNNnnnntgg	2	7585	9215	
!NsiI ATGCAt	2	7626	9503	
!BsgI ctgcac	1	7809		
!BbsI gtcttc	2	7820	8616	
!BlpI GCtnagc	1	8017		
!EspI GCtnagc	1	8017		
!EcoO109I RGgnccy	2	8073	8605	
!Ecl136I GAGctc	1	8080		
!SacI GAGCTc	1	8080		
!End light chain		8122		
!AscI GGcgcgcc	1	8126		
!BssHII Gcgcgc	1	8127		
!RBS2		8147		
!SfiI GGCCNNNNnggcc	1	8207		
!NcoI Ccatgg	1	8218		
!Start 3-23, FR1		8226		
!MfeI Caattg	1	8232		
!BspEI Tccgga	1	8298		
!Start CDR1		8316		
!Statt FR2		8331		
!BstXI CCANNNNNntgg	2	8339	8812	
!EcoNI CCTNNnnnagg	2	8346	8675	
!Start FR3		8373		
!XbaI Tctaga	2	8436	1643	
!AflII Cttaag	1	8480		
!Start CDR3		8520		
!AatII GACGTc	1	8556		
!Start FR4		8562		
!PshAI GACNNnngtc	2	8573	9231	

```
!BstEII Ggtnacc                 1    8579
!Start CH1                           8595
!ApaI GGGCCc                    1    8606
!Bsp120I Gggccc                 1    8606
!PspOMI Gggccc                  1    8606
!AgeI Accggt                    1    8699
!Bsu36I CCtnagg                 2    8770  9509
!End of CH1                          8903
!NotI GCggccgc                  1    8904
!Start His6 tag                      8913
!Start cMyc tag                      8931
!Amber codon                         8982
!NheI Gctagc                    1    8985
!Start M13 III Domain 3              8997
!NruI TCGcga                    1    9106
!BstBI TTcgaa                   1    9197
!EcoRI Gaattc                   1    9200
!XcmI CCANNNNNnnnntgg           1    9215
!BstAPI GCANNNNntgc             1    9337
!SacII CCGCgg                   1    9365
!End IIIstump anchor                 9455
!AvrII Cctagg                   1    9462
!trp terminator                      9470
!SwaI ATTTaaat                  1    9784
!Start gene II                       9850
!BglII Agatct                   1    9936
!-------------------------------------------------------------------
--
     1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc
gcc
!    gene ii continued
    49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat
gta
    97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca
act
   145 gtt aTa tgg aat gaa act tcc aga cac cgt act tta gtt gca tat
tta
   193 aaa cat gtt gag cta cag caT TaT att cag caa tta agc tct aag
cca
   241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc
tct
   289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa
gct
   337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat
ctt
   385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa
gac
   433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa
gca
   481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg
gac
!                          Start gene x, ii continues
   529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act
tct
   577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta
aac
   625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt
tgg
   673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa
ctg
   721 atg aat ctt tct acc tgt aat aat gtt gtt ccg tta gtt cgt ttt
att
```

```
   769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca
gtt
   817 ctt aaa atc gca TAA
!                      End X & II
   832 ggtaattca ca
!
!       M1              E5                  Q10                 T15
   843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act
cgt
!      Start gene V
!
!      S17         S20                 P25                 E30
   891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag
ctt
!
!              V35                 E40                 V45
   939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att
act
!
!           D50                 A55                 L60
   987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt
cat
!                                                   BsrGI...
!      L65                 V70                 S75
R80
  1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac
cgt
!
!                      P85     K87 end of V
  1083 ctg cgc ctc gtt ccg gct aag TAA C
!
  1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg
!      Start gene VII
!
  1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc
!
!                        VII and IX overlap.
!                        ..... S2  V3  L4  V5                S10
  1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttT gcc tct ttc
gtt
!                          End VII
!                        |start IX
!      L13     W15                 G20                 T25
E29
  1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta
atg gaa
!
  1293 act tcc tc
!
!       .... stop of IX, IX and VIII overlap by four bases
  1301 ATG aaa aag tct tta gtc ctc aaa gcc tct gta gcc gtt gct acc
ctc
!      Start signal sequence of viii.
!
  1349 gtt ccg atg ctg tct ttc gct gct gag ggt gac gat ccc gca aaa
gcg
!                                  mature VIII --->
  1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat
gcg
  1445 tgg gcg atg gtt gtt gtc att
  1466 gtc ggc gca act atc ggt atc aag ctg ttt aag
!
```

```
! bases 1499-1539 are probable promoter for iii
  1499 aaa ttc acc tcg aaa gca ! 1515
!       ..........  -35  ..
!
  1517      agc tga taaaccgat acaattaaag gctccttttg
!                       ..... -10   ...
!
  1552 gagccttttt ttt GGAGAt ttt ! S.D. uppercase, there may be 9 Ts
!
!           <------ III signal sequence ----------------------------
>
!             M   K   K   L   L   F   A   I   P   L   V   V   P   F
  1574 caac GTG aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc !
1620
!
!       Y   S   G   A   A   E   S   H   L   D   G   A
  1620 tat tct ggc gCG GCC Gaa tca caT CTA GAc ggc gcc
!                   EagI....         XbaI....
!
! Domain 1 ------------------------------------------------------
--
!           A   E   T   V   E   S   C   L   A
  1656     gct gaa act gtt gaa agt tgt tta gca
!
!       K   S   H   T   E   I   S   F   T   N   V   W   K   D   D   K
 T
  1683 aaA Tcc cat aca gaa aat tca ttt aCT AAC GTC TGG AAA GAC GAC
AAA ACt
!
!       L   D   R   Y   A   N   Y   E   G   S   L   W   N   A   T   G
 V
  1734 tta gat cgt tac gct aac tat gag ggC tgt ctg tgG AAT GCt aca
ggc gtt
!                                                       BsmI....
!
!       V   V   C   T   G   D   E   T   Q   C   Y   G   T   W   V   P
 I
  1785 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT
cct att
!
!       G   L   A   I   P   E   N
  1836 ggg ctt gct atc cct gaa aat
!
! L1 linker -----------------------------------
!       E   G   G   G   S   E   G   G   G   S
  1857 gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!       E   G   G   G   S   E   G   G   G   T
  1887 gag ggt ggc ggt tct gag ggt ggc ggt act
!
!  Domain 2 -----------------------------------
  1917 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat
atc aac
  1968 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct
aat cct
  2019 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt
cag aat
!                     BseRI..
  2070 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc
act
  2118 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act
cct
```

```
  2166 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC
AGA
!
AlwNI
  2214 GAC TGc gct ttc cat tct ggc ttt aat gaG gat TTa ttT gtt tgt
gaa
!       AlwNI
  2262 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct
!
  2307 ggc ggc ggc tct
!  start L2 ------------------------------------------------------
----
  2319 ggt ggt ggt tct
  2331 ggt ggc ggc tct
  2343 gag ggt ggt ggc tct gag gga ggc ggt tcc
  2373 ggt ggt ggc tct ggt    ! end L2
!
! Many published sequences of M13-derived phage have a longer linker
! than shown here by repeats of the EGGGS motif two more times.
!
! Domain 3 ------------------------------------------------------
----
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  2388 tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg
gct
!
!       M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
  2436 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa
ggc
!
!       K   L   D   S   V   A   T   D   Y   G   A   A   M   D   G   F
  2484 aaa ctt gat tct gtc gct act gat tac ggt gct gct atc gat ggt
ttc
!
!       I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  2532 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt
gat
!
!       F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  2580 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat
aat
!
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  2628 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct
caa
!
!       S   V   E   C   R   P   F   V   F   G   A   G   K   P   Y   E
  2676 tcg gtt gaa tgt cgc cct ttt gtc ttt Ggc gct ggt aaa cca tat
gaa
!
!       F   S   I   D   C   D   K   I   N   L   F   R
  2724 ttt tct att gat tgt gac aaa ata aac tta ttc cgt
!                                                   End Domain 3
!
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V
F140
  2760 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta
ttt
!      start transmembrane segment
!
!       S   T   F   A   N   I   L
  2808 tct acg ttt gct aac ata ctg
```

```
!
!       R   N   K   E   S
  2829 cgt aat aag gag tct TAA ! stop of iii
!     Intracellular anchor.
!
!           M1  P2  V   L  L5   G   I   P   L  L10  L   R   F   L
G15
  2847  tc ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc
ggt
!           Start VI
!
  2894 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa
aag
  2942 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt
att
  2990 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc
gct
  3038 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg
tct
  3086 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct
att
  3134 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg
gat
!
!                 M1  A2  V3      F5                  L10          G13
  3182 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga
!       end VI   Start gene I
!
!       K   T   L   V   S   V   G   K   I   Q   D   K   I   V   A
  3228 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct
!
!       G   C   K   I   A   T   N   L   D   L   R   L   Q   N   L
  3273 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc
!
!       P   Q   V   G   R   F   A   K   T   P   R   V   L   R   I
  3318 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata
!
!       P   D   K   P   S   I   S   D   L   L   A   I   G   R   G
  3363 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt
!
!       N   D   S   Y   D   E   N   K   N   G   L   L   V   L   D
  3408 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat
!
!       E   C   G   T   W   F   N   T   R   S   W   N   D   K   E
  3453 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa
!
!       R   Q   P   I   I   D   W   F   L   H   A   R   K   L   G
  3498 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta gga
!
!       W   D   I   I   F   L   V   Q   D   L   S   I   V   D   K
  3543 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa
!
!       Q   A   R   S   A   L   A   E   H   V   V   Y   C   R   R
  3588 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt
!
!       L   D   R   I   T   L   P   F   V   G   T   L   Y   S   L
  3633 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt
!
!       I   T   G   S   K   M   P   L   P   K   L   H   V   G   V
  3678 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt
!
!       V   K   Y   G   D   S   Q   L   S   P   T   V   E   R   W
```

```
 3723 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg
!
!       L   Y   T   G   K   N   L   Y   N   A   Y   D   T   K   Q
 3768 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag
!
!       A   F   S   S   N   Y   D   S   G   V   Y   S   Y   L   T
 3813 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg
!
!       P   Y   L   S   H   G   R   Y   F   K   P   L   N   L   G
 3858 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt
!
!       Q   K   M   K   L   T   K   I   Y   L   K   K   F   S   R
 3903 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc
!
!       V   L   C   L   A   I   G   F   A   S   A   F   T   Y   S
 3948 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt
!
!       Y   I   T   Q   P   K   P   E   V   K   K   V   V   S   Q
 3993 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag
!
!       T   Y   D   F   D   K   F   T   I   D   S   S   Q   R   L
 4038 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt
!
!       N   L   S   Y   R   Y   V   F   K   D   S   K   G   K   L
 4083 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA
!                                                           PacI
!
!       I   N   S   D   D   L   Q   K   Q   G   Y   S   L   T   Y
 4128 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat
!    PacI
!
!       i   I   D   L   C   T   V   S   I   K   K   G   N   S   N   E
!     iv                                                        M1  K
 4173    att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa
!                                                              Start
IV
!
!     i    I   V   K   C   N   .End of I
!     iv     L3  L   N5' V   I7  N    F  V10
 4218    att gtt aaa tgt aat TAA T TTT GTT
!  IV continued.....
 4243 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa
atg
 4291 aat aat tcg cct ctg cgc gat ttt gta act tgg tat tca aag caa
tca
 4339 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act
gta
 4387 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att
tct
 4435 gtt tta cgt gcA aat aat ttt gat atg gtA ggt tcT aAC cct tcc
atT
 4483 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg
cca
 4531 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt
ggt
 4579 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att
aat
 4627 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta
aag
 4675 tct aat act tct aaa tcc tca aat gta tta tct att gac ggc tct
aat
 4723 cta tta gtt gtt agt gcT cct aaa gat att tta gat aac ctt cct
```

```
caa
  4771 ttc ctt tcA act gtt gat ttg cca act gac cag ata ttg att gag
ggt
  4819 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt
gct
  4867 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac
cgc
  4915 ctc acc tct gtt tta tct tct gct ggt ggt tcg ttc ggt att ttt
aat
  4963 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc
cat
  5011 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag
aag
  5059 ggt tct atc tct gtT GGC CAg aat gtc cct ttt att act ggt cgt
gtg
!                        MscI....
  5107 act ggt gaa tct gcc aat gta aat aat cca ttt cag acg att gag
cgt
  5155 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct
ggc
  5203 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt
tct
  5251 tct act cag gca agt gat gtt att act aat caa aga agt att gct
aca
  5299 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc
act
  5347 gat tat aaa aac act tct caG gat tct ggc gta ccg ttc ctg tct
aaa
  5395 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcT aac
gag
  5443 gaa agc acg tta tac gtg ctc gtc aaa gca acc ata gta cgc gcc
ctg
  5491 TAG cggcgcatt
!      End IV
  5503 aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca
gcgccctagc
  5563 gcccgctcct ttcgctttct tcccttcctt tctcgccacg ttcGCCGGCt
ttccccgtca
!                                                      NgoMI.
  5623 agctctaaat cgggggctcc ctttagggtt ccgatttagt gctttacggc
acctcgaccc
  5683 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tcgccctgat
agacggtttt
!                                    DraIII....
  5743 tcgccctttG ACGTTGGAGT Ccacgttctt taatagtgga ctcttgttcc
aaactggaac
!               DrdI.........
  5803 aacactcaac cctatctcgg gctattcttt tgatttataa gggattttgc
cgatttcgga
  5863 accaccatca aacaggattt tcgcctgctg gggcaaacca gcgtggaccg
cttgctgcaa
  5923 ctctctcagg gccaggcggt gaagggcaat CAGCTGttgc cCGTCTCact
ggtgaaaaga
!                                          PvuII.      BsmBI.
  5983 aaaaccaccc tGGATCC  AAGCTT
!                  BamHI   HindIII (1/2)
!                  Insert carrying bla gene
  6006    gcaggtg gcacttttcg gggaaatgtg cgcggaaccc
  6043 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga
caataaccct
!                                              BciVI
  6103 gataaatgct tcaataatat tgaaaaAGGA AGAgt
```

```
!                                        RBS.?...
!       Start bla gene
  6138 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg
gca ttt
  6189 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa
gat gct
  6240 gaa gat cag ttg ggC gcA CTA GTg ggt tac atc gaa ctg gat ctc
aac agc
!                                  SpeI....
!                             ApaLI & BssSI Removed
  6291 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg
atg agc
  6342 act ttt aaa gtt ctg cta tgt GGC GcG Gta tta tcc cgt att gac
gcc ggg
  6393 caa gaG CAA CTC GGT CGc cgC ATA cAC tat tct cag aat gac ttg
gtt gAG
!              BcgI...........
 ScaI
  6444 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta
aga gaa
!      ScaI.
  6495 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac
tta ctt
  6546 ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg cac
aac atg
!                PvuI....
  6597 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat
gaa gcc
  6648 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg Gca
aca acg
  6699 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg
caa caa
!       FspI....
!
  6750 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg
cgc tcg
  6801 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt
gag cgt
!      BglI.........
  6852 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc
tcc cgt
!       BsaI....
  6903 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa
cga aat
!                                 AhdI..........
  6954 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA
ctgt
!                                                               stop
  7003 cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt
taatttaaaa
  7063 ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa
cgtgagtttt
  7123 cgttccactg tacgtaagac cccc
  7147 AAGCTT   GTCGAC tgaa tggcgaatgg cgctttgcct
!      HindIII  SalI..
!      (2/2)    HincII
  7183 ggtttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt
!
! Start of Fab-display cassette, the Fab DSR-A05, selected for
! binding to a protein antigen.
!
  7233 CCTGAcG CTCGAG
```

```
!      xBsu36I XhoI..
!
! PlacZ promoter is in the following block
!
5   7246                                cgcaacgc aattaatgtg agttagctca
    7274    ctcattaggc accccaggct ttacacttta tgcttccggc tcgtatgttg
    7324    tgtggaattg tgagcggata acaatttcac acaggaaaca gctatgacca
    7374    tgattacgCC AagcttTGGa gccttttttt tggagatttt caac
!                   PflMI.......
10  !                  Hind3. (there are 3)
! Gene iii signal sequence:
!           1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!           M   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
    7418    gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
15  !
!           16  17  18          Start light chain (L20:JK1)
!            S   H   S   A   Q   D   I   Q   M   T   Q   S   P   A
    7463    tct cac aGT GCA Caa gac atc cag atg acc cag tct cca gcc
!                   ApaLI...
20  !                  Sequence supplied by extender.....
!
!                T   L   S   L
    7505         acc ctg tct ttg
!
25  !           S   P   G   E   R   A   T   L   S   C   R   A   S   Q   G
    7517    tct cca ggg gaa aga gcc acc ctc tcc tgc agg gcc agt cag Ggt
!
!           V   S   S   Y   L   A   W   Y   Q   Q   K   P   G   Q   A
    7562    gtt agc agc tac tta gcc tgg tac cag cag aaa cct ggc cag gct
30  !
!           P   R   L   L   I   Y   D   A   S   S   R   A   T   G   I
    7607    ccc agg ctc ctc atc tat gAt gca tcc aAc agg gcc act ggc atc
!
!           P   A   R   F   S   G   S   G   P   G   T   D   F   T   L
35  7652    cca gCc agg ttc agt ggc agt ggg Cct ggg aca gac ttc act ctc
!
!           T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C
    7697    acc atc agc agC ctA gag cct gaa gat ttt gca gtT tat tac tgt
!
40  !           Q   Q   R   S   W   H   P   W   T   F   G   Q   G   T   R
    7742    cag cag CGt aAc tgg cat ccg tgg ACG TTC GGC CAA GGG ACC AAG
!
!           V   E   I   K   R   T   V   A   A   P   S   V   F   I   F
    7787    gtg gaa atc aaa cga act gtg gCT GCA Cca tct gtc ttc atc ttc
45  !                                   BsgI....
!
!           P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V
    7832    ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg
!
50  !           C   L   L   N   N   F   Y   P   R   E   A   K   V   Q   W
    7877    tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg
!
!           K   V   D   N   A   L   Q   S   G   N   S   Q   E   S   V
    7922    aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc
55  !
!           T   E   R   D   S   K   D   S   T   Y   S   L   S   S   T
    7967    aca gag cgg gac agc aag gac agc acc tac agc ctc agc agc acc
!
!           L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A
60  8012    ctg acG CTG AGC aaa gca gac tac gag aaa cac aaa gtc tac gcc
!                EspI.....
!
```

```
!          C   E   V   T   H   Q   G   L   S   S   P   V   T   K   S
  8057     tgc gaa gtc acc cat cag ggc ctG AGC TCg ccc gtc aca aag agc
!                                       SacI....
!
!          F   N   R   G   E   C   .   .
  8102     ttc aac agg gga gag tgt taa taa
!
  8126         GGCGCG CCaattctat ttcaaGGAGA cagtcata
!              AscI.....              RBS2.
!
!           PelB signal sequence------(22 codons)----->
!            1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!           M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
  8160     atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc
!
!          ...PelB signal------------> Start VH, FR1----------------->
!           16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!           A   A   Q   P   A   M   A   E   V   Q   L   L   E   S   G
  8205     gcG GCC cag ccG GCC atg gcc gaa gtt CAA TTG tta gag tct ggt
!            SfiI.............                MfeI...
!                           NcoI....
!
!           31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!           G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
  8250     ggc ggt ctt gtt cag cct ggt ggt tct tta cgt ctt tct tgc gct
!
!          ...FR1--------------------> CDR1--------------> FR2--------
>
!           46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!           A   S   G   F   T   F   S   T   Y   E   M   R   W   V   R
  8295     gct TCC GGA ttc act ttc tct act tac gag atg cgt tgg gtt cgC
!              BspEI..
BstXI...
!
!           FR2---------------------------------------> CDR2 ----------
>
!           61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!           Q   A   P   G   K   G   L   E   W   V   S   Y   I   A   P
  8340     CAa gct ccT GGt aaa ggt ttg gag tgg gtt tct tat atc gct cct
!  BstXI.............
!
!         ...CDR2---------------------------------------------> FR3----
>
!           76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!           S   G   G   D   T   A   Y   A   D   S   V   K   G   R   F
  8385     tct ggt ggc gat act gct tat gct gac tcc gtt aaa ggt cgc ttc
!
!           91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!           T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
  8430     act atc TCT AGA gac aac tct aag aat act ctc tac ttg cag atg
!                XbaI...
!                Supplied by extender------------------------------
!
!           ----------------------------------------FR3------------->
!          106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!           N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   R
  8475     aac agC TTA AGg gct gag gac act gca gtc tac tat tgt gcg agg
!                 AflII...
!           from extender------------------------------->
!
```

```
!         CDR3---------------------------------------------------->
FR4-->
!         121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!          R   L   D   G   Y   I   S   Y   Y   Y   G   M   D   V   W
  8520    agg ctc gat ggc tat att tcc tac tac tac ggt atg GAC GTC tgg
!                                                       AatII..
!
!         136 137 138 139 140 141 142 143 144 145
!          G   Q   G   T   T   V   T   V   S   S
  8565    ggc caa ggg acc acG GTC ACC gtc tca agc
!                       BstEII...
!
!          CH1 of IgG1---------->
!           A   S   T   K   G   P   S   V   F   P   L   A   P   S   S
  8595     gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc
tcc
!
!           K   S   T   S   G   G   T   A   A   L   G   C   L   V   K
  8640    ' aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc
aag
!
!           D   Y   F   P   E   P   V   T   V   S   W   N   S   G   A
  8685     gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc
gcc
!
!           L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S
  8730     ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tCC
TCA
!
Bsu36I....
!
!           G   L   Y   S   L   S   S   V   V   T   V   P   S   S   S
  8775     GGa ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc
agc
!  Bsu36I....
!
!           L   G   T   Q   T   Y   I   C   N   V   N   H   K   P   S
  8820     ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc
agc
!
!           N   T   K   V   D   K   K   V   E   P   K   S   C   A   A
  8865     aac acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt GCG
GCC
!
NotI......
!
!           A   H   H   H   H   H   H   G   A   A   E   Q   K   L   I
  8910     GCa cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc
atc
!  ..NotI....  H6 tag.................. Myc-
Tag.........................
!
!           S   E   E   D   L   N   G   A   A   q   A   S   S   A
  8955     tca gaa gag gat ctg aat ggg gcc gca tag GCT AGC tct gct
!          Myc-Tag...................          ... NheI...
!                                          Amber
!
! III'stump
!
! Domain 3 of III ----------------------------------------------------
----
!
```

```
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  8997 agt ggc gac ttc gac tac gag aaa atg gct aat gcc aac aaa GGC
GCC
!      tcc   t   t   t   t   t   a   g       a   c   t   t   g   g
t !W.T.
!
KasI...(2/4)
!
!       M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
  9045 atG ACT GAG AAC GCT GAC GAG aat gct ttg caa agc gat gcc aag
ggt
!            c   a   t   c   t   a   c   g c a   g tct   c   t   a
c !W.T.
!
!       K   L   D   S   V   A   T   D   Y   G   A   A   I   D   G   F
  9093 aag tta gac agc gTC GCG Acc gac tat GGC GCC gcc ATC GAc ggc
ttt
!        a c t   t tct       t   t   t   c   t   t   t       t   t
c !W.T.
!                        NruI....           KasI...(3/4)
!
!       I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  9141 atc ggc gat gtc agt ggt tTG GCC Aac ggc aac gga gcc acc gga
gac
!        t   t   c   t tcc   c c t   t   t   t   t   t   t   t   t
t !W.T.
!                                MscI....(3/3)
!
!       F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  9189 ttc GCA GGT tcG AAT TCt cag atg gcC CAG GTT GGA GAT GGg gac
aac
!        t   t   c   t       c   a       t   a   c   t   c   t   t
t !W.T.
!          BspMI.. (2/2)                 XcmI.................
!                    EcoRI...
!
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  9237 agt ccg ctt atg aac aac ttt aga cag tac ctt ccg tct ctt ccg
cag
!      tca   t t a       t   t   c c t   a   t t a   t   c   c   t
a !W.T.
!
!       S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
  9285 agt gtc gag tgc cgt cca ttc gtt ttc tct gcc ggc aag cct tac
gag
!      tcg   t   a   t   c   t   t   c   t agc   t   t   a   a   t
a !W.T.
!
!       F   S   I   D   C   D   K   I   N   L   F   R
  9333 ttc aGC Atc gac TGC gat aag atc aat ctt ttC CGC
!        t tct   t   t   t   c   a   a   c t a   c   t  !W.T.
!           BstAPI........                       SacII...
!                                                  End Domain 3
!
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V   F
  9369 GGc gtt ttc gct ttc ttg cta tac gtc gct act ttc atg tac gtt
ttc
!        t   c   t   g   t c t t a   t   t   c   c   t       t   a
t !W.T.
!       start transmembrane segment
!
!       S   T   F   A   N   I   L     R   N   K   E   S
```

```
  9417 aGC ACT TTC GCC AAT ATT TTA   Cgc aac aaa gaa agc
!      tct   g   t   t   c   a c g     t   t   g   g tct !W.T.
!                                   Intracellular anchor.
!
!              .   .
  9453         tag tga tct CCT AGG
!                        AvrII..
!
  9468 aag ccc gcc taa tga gcg ggc ttt ttt ttt ct  ggt
!        | Trp terminator                      |
!
! End Fab cassette
!
  9503   ATGCAT CCTGAGG  ccgat actgtcgtcg tcccctcaaa ctggcagatg
!        NsiI.. Bsu36I.(3/3)
  9551 cacggttacg atgcgcccat ctacaccaac gtgacctatc ccattacggt
caatccgccg
  9611 tttgttccca cggagaatcc gacgggttgt tactcgctca catttaatgt
tgatgaaagc
  9671 tggctacagg aaggccagac gcgaattatt tttgatggcg ttcctattgg
ttaaaaaatg
  9731 agctgattta acaaaaattt aaTgcgaatt ttaacaaaat attaacgttt
acaATTTAAA
!
SwaI...
  9791 Tatttgctta tacaatcttc ctgtttttgg ggcttttctg attatcaacc
GGGGTAcat
  9850 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt
gtt tgc
!      Start gene II
  9901 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca
aaa ata
!                                                    BglII...
  9952 gct acc ctc tcc ggc atT aat tta tca gct aga acg gtt gaa tat
cat att
 10003 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct
tta cct
 10054 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa
aat ttt
 10105 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag
ggt cat
 10156 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta
ttg ctt
 10207 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt !
! gene II continues
!------------------------ End of Table -----------------------------
-
```

Table 37: DNA seq of w.t. M13 gene iii

```
        1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
       fM   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
1579  gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
      Signal sequence.....................................

       16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
        S   H   S   A   E   T   V   E   S   C   L   A   K   P   H
1624 tct cac tcc gct gaa act gtt gaa agt tgt tta gca aaa ccc cat
Signal sequence> Domain 1-------------------------------------

       31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
        T   E   N   S   F   T   N   V   W   K   D   D   K   T   L
1669 aca gaa aat tca ttt act aac gtc tgg aaa gac gac aaa act tta
     Domain 1-----------------------------------------------

       46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
        D   R   Y   A   N   Y   E   G   C   L   W   N   A   T   G
1714 gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc
                                            BsmI....
     Domain 1-----------------------------------------------

       61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
        V   V   V   C   T   G   D   E   T   Q   C   Y   G   T   W
1759 gtt gta gtt tgt act ggt gac gaa act cag tgt tac ggt aca tgg
     Domain 1-----------------------------------------------

       76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
        V   P   I   G   L   A   I   P   E   N   E   G   G   G   S
1804 gtt cct att ggg ctt gct atc cct gaa aat gag ggt ggt ggc tct
     Domain 1----------------------------> Linker 1-----------

       91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
        E   G   G   G   S   E   G   G   G   S   E   G   G   G   T
1849 gag ggt ggc ggt tct gag ggt ggc ggt tct gag ggt ggc ggt act
     Linker 1----------------------------------------------->

      106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
        K   P   P   E   Y   G   D   T   P   I   P   G   Y   T   Y
1894 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat
     Domain 2-----------------------------------------------

      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
        I   N   P   L   D   G   T   Y   P   P   G   T   E   Q   N
1939 atc aac cct ctc gac ggc act taT CCG CCt ggt act gag caa aac
                                    EciI....
     Domain 2-----------------------------------------------

      136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
        P   A   N   P   N   P   S   L   E   E   S   Q   P   L   N
1984 ccc gct aat cct aat cct tct ctt GAG GAG tct cag cct ctt aat
                                      BseRI..
     Domain 2-----------------------------------------------

      151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
        T   F   M   F   Q   N   N   R   F   R   N   R   Q   G   A
2029 act ttc atg ttt cag aat aat agg ttc cga aat agg cag ggg gca
     Domain 2-----------------------------------------------

      166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
        L   T   V   Y   T   G   T   V   T   Q   G   T   D   P   V
```

```
  2074 tta act gtt tat acg ggc act gtt act caa ggc act gac ccc gtt
!      Domain 2-----------------------------------------------
!
!      181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
5 !     K   T   Y   Y   Q   Y   T   P   V   S   S   K   A   M   Y
  2119 aaa act tat tac cag tac act cct gta tca tca aaa gcc atg tat
!      Domain 2-----------------------------------------------
!
!      196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
10 !    D   A   Y   W   N   G   K   F   R   D   C   A   F   H   S
  2164 gac gct tac tgg aac ggt aaa ttC AGa gaC TGc gct ttc cat tct
!                                    AlwNI.......
!      Domain 2-----------------------------------------------
!
15 !    211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!       G   F   N   E   D   P   F   V   C   E   Y   Q   G   Q   S
  2209 ggc ttt aat gaG GAT CCa ttc gtt tgt gaa tat caa ggc caa tcg
!               BamHI...
!      Domain 2-----------------------------------------------
20 !
!      226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!       S   D   L   P   Q   P   P   V   N   A   G   G   G   S   G
  2254 tct gac ctg cct caa cct cct gtc aat gct ggc ggc ggc tct ggt
!      Domain 2----------------------------> Linker 2-----------
25 !
!      241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!       G   G   S   G   G   G   S   E   G   G   G   S   E   G   G
  2299 ggt ggt tct ggt ggc ggc tct gag ggt ggt ggc tct gag ggt ggc
!      Linker 2-----------------------------------------------
30 !
!      256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!       G   S   E   G   G   G   S   E   G   G   G   S   G   G   G
  2344 ggt tct gag ggt ggc ggc tct gag gga ggc ggt tcc ggt ggt ggc
!      Linker 2-----------------------------------------------
35 !
!      271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!       S   G   S   G   D   F   D   Y   E   K   M   A   N   A   N
  2389 tct ggt tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat
!Linker 2>     Domain 3-------------------------------------------
40 !
!      286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!       K   G   A   M   T   E   N   A   D   E   N   A   L   Q   S
  2434 aag ggg gct atg acc gaa aat gcc gat gaa aac gcg cta cag tct
!      Domain 3-----------------------------------------------
45 !
!      301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
!       D   A   K   G   K   L   D   S   V   A   T   D   Y   G   A
  2479 gac gct aaa ggc aaa ctt gat tct gtc gct act gat tac ggt gct
!      Domain 3-----------------------------------------------
50 !
!      316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!       A   I   D   G   F   I   G   D   V   S   G   L   A   N   G
  2524 gct atc gat ggt ttc att ggt gac gtt tcc ggc ctt gct aat ggt
!      Domain 3-----------------------------------------------
55 !
!      331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!       N   G   A   T   G   D   F   A   G   S   N   S   Q   M   A
  2569 aat ggt gct act ggt gat ttt gct ggc tct aat tcc caa atg gct
!      Domain 3-----------------------------------------------
60 !
```

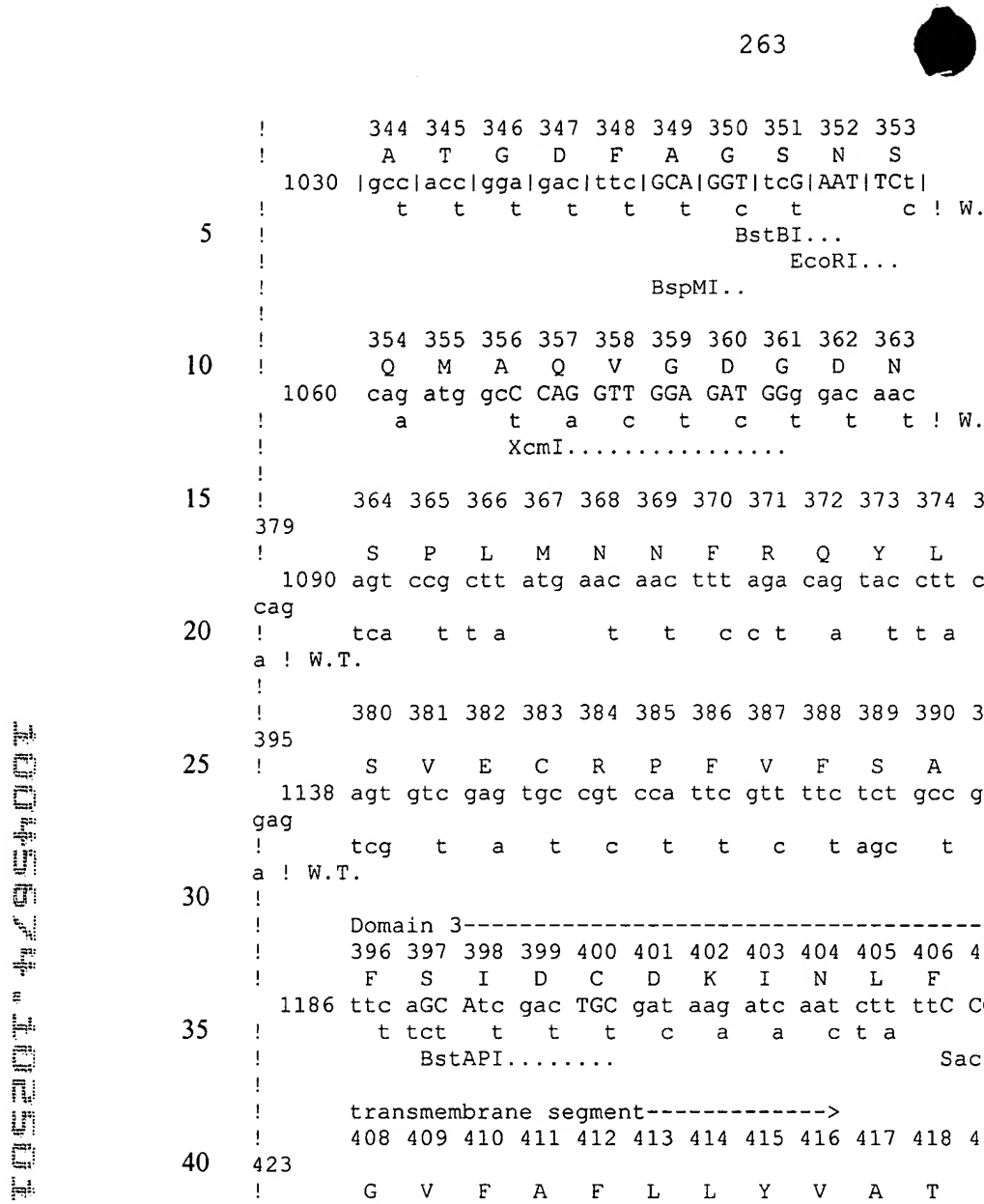
```
!      346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!       Q   V   G   D   G   D   N   S   P   L   M   N   N   F   R
  2614 caa gtc ggt gac ggt gat aat tca cct tta atg aat aat ttc cgt
!      Domain 3-----------------------------------------------------
!
!      361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!       Q   Y   L   P   S   L   P   Q   S   V   E   C   R   P   F
  2659 caa tat tta cct tcc ctc cct caa tcg gtt gaa tgt cgc cct ttt
!      Domain 3-----------------------------------------------------
!
!      376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!       V   F   S   A   G   K   P   Y   E   F   S   I   D   C   D
  2704 gtc ttt agc gct ggt aaa cca tat gaa ttt tct att gat tgt gac
!      Domain 3-----------------------------------------------------
!
!      391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!       K   I   N   L   F   R   G   V   F   A   F   L   L   Y   V
  2749 aaa ata aac tta ttc cgt ggt gtc ttt gcg ttt ctt tta tat gtt
!      Domain 3--------------> Transmembrane segment--------------
!
!      406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
!       A   T   F   M   Y   V   F   S   T   F   A   N   I   L   R
  2794 gcc acc ttt atg tat gta ttt tct acg ttt gct aac ata ctg cgt
!      Transmembrane segment----------------------------------> ICA--
!
!      421 422 423 424 425
!       N   K   E   S   .
  2839 aat aag gag tct taa ! 2853
!      ICA----------->            ICA = intracellular anchor
!
!------------------ End of Table -----------------------------------
-----
```

Table 38: Whole mature III anchor M13-III derived anchor with recoded DNA

```
!
!          1   2   3
!          A   A   A
     1    GCG gcc gca
!         NotI......
!
!          4   5   6   7   8   9  10  11  12  13  14  15  16  17
!          H   H   H   H   H   H   G   A   A   E   Q   K   L   I
    10    cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc atc
!
!         18  19  20  21  22  23  24  25  26  27  28  29
!          S   E   E   D   L   N   G   A   A   .   A   S
    52    tca gaa gag gat ctg aat ggg gcc gca Tag GCT AGC
!                                              NheI...
!
!        30  31  32  33  34  35  36    37  38  39
!         D   I   N   D   D   R   M     A   S   T
    88   GAT ATC aac gat gat cgt atg    gct tct act
! (ON_G37bot) [RC] 5'-c aac gat gat cgt atg gcG CAt Gct gcc gag aca
g-3'
!        EcoRV..
!        Enterokinase cleavage site.
!
!  Start mature III (recoded)  Domain 1 ---->
!              40  41  42  43
!               A   E   T   V
   118         |gcC|gaG|acA|gtC|
!                t   a   t   t ! W.T.
!
!        44  45  46  47  48  49  50  51  52  53  54  55  56  57  58
!         E   S   C   L   A   K   P   H   T   E   N   S   F   T   N
   130  |gaa|TCC|tgC|CTG|GCC|AaG|ccT|caC|acT|gaG|aat|AGT|ttC|aCA|Aat|
!           agt   t t a   a   a   c   t   a   a     tca   t   t   c
! W.T.
!                    MscI....
!
!       59  60  61  62  63  64  65  66  67  68  69  70  71  72  73
!        V   W   K   D   D   K   T   L   D   R   Y   A   N   Y   E
   175 |gtg|TGG|aaG|gaT|gaT|aaG|acC|CtT|gAT|CGA|TaT|gcC|aaT|taC|gaA|
!         c       a   c   c   a   t t a       t   c   t   c   t   g !
W.T.
!                                        BspDI...
!
!       74  75  76  77  78  79  80  81  82  83  84  85  86  87  88
!        G   C   L   W   N   A   T   G   V   V   V   C   T   G   D
   220 |ggC|tgC|TtA|tgg|aat|gcC|ACC|GGC|GtC|gtT|gtC|TGC|ACG|ggC|gaT|
!         t   t c g           t   a       t   a   t   t   t   t   c !
W.T.
!                              SgrAI......         BsgI....
!
!       89  90  91  92  93  94  95  96  97  98  99  100 101 102 103
!        E   T   Q   C   Y   G   T   W   V   P   I   G   L   A   I
   265 |gaG|acA|caA|tgC|taT|ggC|ACG|TGg|gtG|ccG|atA|gGC|TTA|GCC|atA|
!         a   t   g   t   c   t   a       t   t   t   g c t   t   c !
W.T.
!                              PmlI....              BlpI.....
!
```

```
!    Domain 1-----> Linker 1---------------->
!       104 105 106 107 108 109 110 111 112 113 114 115 116 117 118
!        P   E   N   E   G   G   G   S   E   G   G   G   S   E   G
   310 |ccG|gaG|aaC|gaA|ggC|ggC|ggT|AGC|gaA|ggC|ggT|ggC|AGC|gaA|ggC|
!        t   a   t   g   t   t   c tct   g   t   c   t tct   g   t !
W.T.
!
!       Linker 1---------------------> Domain 2--------------->
!       119 120 121 122 123 124 125 126 127 128 129 130 131 132 133
!        G   G   S   E   G   G   G   T   K   P   P   E   Y   G   D
   355 |ggT|GGA|TCC|gaA|ggA|ggT|ggA|acC|aaG|ccG|ccG|gaA|taT|ggC|gaC|
!        c   t   t   g   t   c   t   t   a   t   t   g   c   t   t !
W.T.
!           BamHI..(2/2)
!
!       134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
!        T   P   I   P   G   Y   T   Y   I   N   P   L   D   G   T
   400 |acT|ccG|atA|CCT|GGT|taC|acC|taC|atT|aaT|ccG|TtA|gaT|ggA|acC|
!        a   t   t   g   c   t   t   t   c   c   t c c   c   c   t !
W.T.
!               SexAI....
!
!       149 150 151 152 153 154 155 156 157 158 159 160 161 162 163
!        Y   P   P   G   T   E   Q   N   P   A   N   P   N   P   S
   445 |taC|ccT|ccG|ggC|acC|gaA|caG|aaT|ccT|gcC|aaC|ccG|aaC|ccA|AGC|
!        T   G   t   t   t   g   a   c   c   t   t   t   t   t tct !
W.T.
!
HindIII...
!
!       164 165 166 167 168 169 170 171 172 173 174 175 176 177 178
!        L   E   E   S   Q   P   L   N   T   F   M   F   Q   N   N
   490 |TTA|gaA|gaA|AGC|caA|ccG|TtA|aaC|acC|ttT|atg|ttC|caA|aaC|aaC|
!        c t   G   G tct   g   t c t   t   t   c       t   g   t   t !
W.T.
! HindIII.
!
!       179 180 181 182 183 184 185 186 187 188 189 190 191 192 193
!        R   F   R   N   R   Q   G   A   L   T   V   Y   T   G   T
   535 |CgT|ttT|AgG|aaC|CgT|caA|gGT|GCT|CtT|acC|gTG|TAC|AcT|ggA|acC|
!        a g   c c a   t a g   g   g   a t a   t   t   t   g   c   t !
W.T.
!                                HgiAI...        BsrGI...
!
!       194 195 196 197 198 199 200 201 202 203 204 205 206 207 208
!        V   T   Q   G   T   D   P   V   K   T   Y   Y   Q   Y   T
   580 |gtC|acC|caG|GGT|ACC|gaT|ccT|gtC|aaG|acC|taC|taT|caA|taT|acC|
!        t   t   a   c   t   c   c   t   a   t   t   c   g   c   t !
W.T.
!                   KpnI...
!
!       209 210 211 212 213 214 215 216 217 218 219 220 221 222 223
!        P   V   S   S   K   A   M   Y   D   A   Y   W   N   G   K
   625 |ccG|gtC|TCG|AGt|aaG|gcT|atg|taC|gaT|gcC|taT|tgg|aaT|ggC|aaG|
!        t   a   a tca   a   c       t   c   t   c       c   t   a !
W.T.
!         BsaI....
!             XhoI....
!
!       224 225 226 227 228 229 230 231 232 233 234 235 236 237 238
!        F   R   D   C   A   F   H   S   G   F   N   E   D   P   F
   670 |ttT|CgT|gaT|tgT|gcC|ttT|caC|AGC|ggT|ttC|aaC|gaa|gac|CCt|ttT|
```

```
!         C A a   C   c   t   c   t tct   c   t   t   G   T   a   c !
W.T.
!
!       239 240 241 242 243 244 245 246 247 248 249 250 251 252 253
!        V   C   E   Y   Q   G   Q   S   S   D   L   P   Q   P   P
   715 |gtC|tgC|gaG|taC|caG|ggT|caG|AGT|AGC|gaT|TtA|ccG|caG|ccA|CCG|
!         t   t   a   t   a   c   a tcg tct   c c g   t.  a   t   t !
W.T.
! DrdI.....
AgeI.....
!
!  Domain 2--------> Linker 2---------------------->
!       254 255 256 257 258 259 260 261 262 263 264 265 266 267 268
!        V   N   A   G   G   G   S   G   G   G   S   G   G   G   S
   760  |GTT|AAC|gcG|ggT|ggT|ggT|AGC|ggC|ggA|ggC|AGC|ggC|ggT|ggT|AGC|
!          c   t   t   c   c   c tct   t   t   t tct   t   c   c tct
! W.T.
! AgeI.....
!        HpaI...
!        HincII.
!
!       Linker 2---------------------------------------------->
Domain 3-->
!       269 270 271 272 273 274 275 276 277 278 279 280 281 282 283
!        E   G   G   G   S   E   G   G   G   S   G   G   G   S   G
   805 |gaA|ggC|ggA|ggT|AGC|gaA|ggA|ggT|ggC|AGC|ggA|ggC|ggT|AGC|ggC|
!          g   t   t   c tct   g   t   c   t tct   g   t   c tct   t
! W.T.
!
!       ------------Domain 3------------------->
!       284 285 286 287 288 289 290 291 292 293 294 295 296 297 298
!        S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G
   850 |AGT|ggC|gac|ttc|gac|tac|gag|aaa|atg|gct|aat|gcc|aac|aaa|GGC|
!       tcc   t   t   t   t   t   a   g       a   c   t   t   g   g !
W.T.
!
KasI....
!
!       299 300 301 302 303 304 305 306 307 308 309 310 311 312 313
!        A   M   T   E   N   A   D   E   N   A   L   Q   S   D   A
   895 |GCC|atg|act|gag|aac|gct|gac|gaG|AAT|GCA|ctg|caa|agt|gat|gCC|
!         t       c   a   t   c   t   a   c   g   a   g tct   c   t !
W.T.
!  KasI....                            BsmI....
StyI...
!
!       314 315 316 317 318 319 320 321 322 323 324 325 326 327 328
!        K   G   K   L   D   S   V   A   T   D   Y   G   A   A   I
   940 |AAG|GGt|aag|tta|gac|agc|gTC|GCc|Aca|gac|tat|ggT|GCt|gcc|atc|
!         a   c   a c t   t tct       t   t   t   c           t     !
W.T.
!  StyI......          PflFI......
!
!       329 330 331 332 333 334 335 336 337 338 339 340 341 342 343
!        D   G   F   I   G   D   V   S   G   L   A   N   G   N   G
   985 |gac|ggc|ttt|atc|ggc|gat|gtc|agt|ggt|ctg|gct|aac|ggc|aac|gga|
!         t   t   c   t   t   c   t tcc   c c t       t   t   t   t !
W.T.
!
```



```
!       344 345 346 347 348 349 350 351 352 353
!        A   T   G   D   F   A   G   S   N   S
  1030 |gcc|acc|gga|gac|ttc|GCA|GGT|tcG|AAT|TCt|
!         t   t   t   t   t   t   c   t       c ! W.T.
!                                 BstBI...
!                                     EcoRI...
!                             BspMI..
!
!       354 355 356 357 358 359 360 361 362 363
!        Q   M   A   Q   V   G   D   G   D   N
  1060  cag atg gcC CAG GTT GGA GAT GGg gac aac
!         a       t   a   c   t   c   t   t   t ! W.T.
!                 XcmI.................
!
!      364 365 366 367 368 369 370 371 372 373 374 375 376 377 378
379
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  1090 agt ccg ctt atg aac aac ttt aga cag tac ctt ccg tct ctt ccg
cag
!      tca   t t a       t   t   c c t   a   t t a   t   c   c   t
a ! W.T.
!
!      380 381 382 383 384 385 386 387 388 389 390 391 392 393 394
395
!       S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
  1138 agt gtc gag tgc cgt cca ttc gtt ttc tct gcc ggc aag cct tac
gag
!      tcg   t   a   t   c   t   t   c   t agc   t   t   a   a   t
a ! W.T.
!
!      Domain 3--------------------------------------->
!      396 397 398 399 400 401 402 403 404 405 406 407
!       F   S   I   D   C   D   K   I   N   L   F   R
  1186 ttc aGC Atc gac TGC gat aag atc aat ctt ttC CGC
!         t tct   t   t   t   c   a   a   c t a       t
!            BstAPI........                       SacII...
!
!      transmembrane segment------------->
!      408 409 410 411 412 413 414 415 416 417 418 419 420 421 422
423
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V   F
  1222 GGc gtt ttc gct ttc ttg cta tac gtc gct act ttc atg tac gtt
ttc
!        t   c   t   g   t c t t a   t   t   c   c   t       t   a
t ! W.T.
!
!      424 425 426 427 428 429 430   431 432 433 434 435
!       S   T   F   A   N   I   L     R   N   K   E   S
  1270 aGC ACT TTC GCC AAT ATT TTA   Cgc aac aaa gaa agc
!      tct   g   t   t   c   a c g     t   t   g   g tct ! W.T.

!                                    Intracellular anchor.
!
!
!                .   .
  1306          tag tga tct CCT AGG
!                           AvrII..
!
  1321 aag ccc gcc taa tga gcg ggc ttt ttt ttt ct  ggt
!        | Trp terminator                      |
!
! End Fab cassette
!--------------------------- End of Table -----------------------
```

Table 39: ONs to make deletions in III

```
! ONs for use with NheI
!
      N
(ON_G29bot)                              5'-c gTT gAT ATc gcT Agc cTA Tgc-
3'   ! 22
! this is the reverse complement of 5'-gca tag gct agc gat atc aac g-
3'
!                                                    NheI... scab.....
(ON_G104top) 5'-g|ata|ggc|tta|gcT|aGC|ccg|gag|aac|gaa|gg-3'
   ! 30
!               Scab.....NheI... 104 105 106 107 108
(ON_G236top) 5'-c|ttt|cac|agc|ggt|ttc|GCT|AGC|gac|cct|ttt|gtc|tgc-3'
   ! 37
!                                      NheI... 236 237 238 239 240
(ON_G236tCS) 5'-c|ttt|cac|agc|ggt|ttc|GCT|AGC|gac|cct|ttt|gtc|Agc-
!                                      NheI... 236 237 238 239 240
               gag|tac|cag|ggt|c-3'
   ! 50

! ONs for use with SphI G CAT Gc
(ON_X37bot)       5'-gAc TgT cTc ggc Agc ATg cgc cAT Acg ATc ATc gTT
g-3' ! 37
!                        N   D   D   R   M   A   H   A
!(ON_X37bot)=[RC] 5'-c aac gat gat cgt atg gcG CAt Gct gcc gag aca
gtc-3'
!                                                 SphI....Scab.....
(ON_X104top) 5'-g|gtG ccg|ata|ggc|ttG|CAT|GCa|ccg|gag|aac|gaa|gg-3'
   ! 36
!               Scab.............SphI....  104 105 106 107 108
(ON_X236top) 5'-c|ttt|cac|agc|ggt|ttG|CaT|gCa|gac|cct|ttt|gtc|tgc-3'
   ! 37
!                                    SphI....  236 237 238 239 240
(ON_X236tCS) 5'-c|ttt|cac|agc|ggt|ttG|CaT|gCa|gac|cct|ttt|gtc|Agc-
!                                      NheI... 236 237 238 239 240
               gag|tac|cag|ggt|c-3'
   ! 50
```

Table 40: Phage titers and enrichments of selections with a DY3F31-based human Fab library

	Input (total cfu)	Output (total cfu)	Output/input ratio
R1-ox selected on phOx-BSA	$4{,}5 \times 10^{12}$	$3{,}4 \times 10^{5}$	$7{,}5 \times 10^{-8}$
R2-Strep selected on Strep-beads	$9{,}2 \times 10^{12}$	3×10^{8}	$3{,}3 \times 10^{-5}$

Table 41: Frequency of ELISA positives in DY3F31-based Fab libraries

	Anti-M13 HRP	9E10/RAM-HRP	Anti-CK/CL Gar-HRP
R2-ox (with IPTG induction)	18/44	10/44	10/44
R2-ox (without IPTG)	13/44	ND	ND
R3-strep (with IPTG)	39/44	38/44	36/44
R3-strep (without IPTG)	33/44	ND	ND